

THE AMERICAN JOURNAL
OF PATHOLOGY

THE AMERICAN JOURNAL OF PATHOLOGY

*Official Publication of
The American Association of Pathologists and Bacteriologists*

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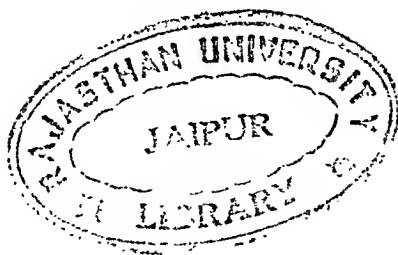
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VOLUME XI

1935



BOSTON
MASSACHUSETTS
U. S. A.

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PRINTED AT THE HARVARD UNIVERSITY PRESS
CAMBRIDGE, MASS., U. S. A.

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VOLUME XI

JANUARY, 1935

NUMBER 1

THE PATHOLOGY OF THE PARATHYROID GLAND IN HYPERPARATHYROIDISM *

A STUDY OF 25 CASES

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INTRODUCTION

De Santi ⁴⁵ was the first to recognize a tumor of parathyroid origin in 1900, twenty years after the discovery of the glands in 1880 by Sandström.¹²⁴ During the next two decades scattered tumors were reported, usually from postmortem examinations. Although the association of these tumors with the clinical syndrome of von Recklinghausen's osteitis fibrosa generalisata had long been recognized, the cure of the disease following the surgical removal of an enlarged parathyroid gland by Mandl in 1925 ⁹³ stimulated greatly increased interest in hyperparathyroidism. The rapidly growing list of case reports, numbering 160 at the time of writing, has added greatly to our knowledge of the condition. No one investigator, however, has hitherto had the opportunity to study more than a few cases, the largest series and the best histological studies to date emanating from Bergstrand with 6 cases,²⁰⁻²⁴ and from Hunter and Turnbull with 5.^{79, 80} The recognition by Albright and co-workers ^{3, 5} that systematic studies of the calcium and phosphorus metabolism in all cases of renal stones would unearth a significant proportion of cases of hyperparathyroidism, added to the already keen interest in the clinical syndrome dating from the work of Albright, Aub, Bauer and

* Received for publication August 11, 1934.

co-workers,^{4, 6, 16, 118} Hannon *et al.*,^{68, 97} has provided us with 25 cases for study. Within this group we have been able to find examples of nearly every type of the disease recorded in the literature, so that an effort at classification seems justified.

For purposes of orientation, since the classification, and still more the nomenclature, of the types of parathyroid cells is regrettably confused, a study of the normal glands seemed necessary. Although it provides little that can be considered new, we feel that our description of the normal will provide the reader with a base line from which he can more adequately evaluate our descriptions of the diseased glands. We therefore include it in brief form.

THE NORMAL PARATHYROID GLAND

In 1898 Welsh,¹⁵³ after examining the parathyroid glands from 40 human autopsies, published an accurate description of the histology of the normal gland, to which little of fundamental importance has been added. He recognized for the first time the "oxyphil" cell, which he distinguished clearly from the predominant "principal" or "chief" cell. The latter he separated into four subtypes, basing his classification partly on the morphology but more on their arrangement. He believed that the least specialized cell was what is now called the "water-clear" or "wasserhelle" cell, and that as the cell became more specialized it reached the stage of a true chief cell. The arrangement of both the oxyphil and chief cells varied from large continuous masses to anastomosing and then branching columns, and finally to cords a single cell wide. True acini were occasionally, but rarely, found and when present often contained colloid-like secretion.

During the past thirty years many anatomists have studied the normal histology of the parathyroid gland, describing various types of cells. One difficulty in comprehending this normal histology is the nomenclature. Von Verebélý¹⁴⁷ describes chief, vacuolated, small and large oxyphil cells; Getzowa,⁵⁶ wasserhelle, rosarote and oxyphil cells; Hunter and Turnbull,⁸⁰ principal and pale and dark oxyphil cells; Erdheim,⁵⁰ a large pale and a dark oxyphil cell; Kurokawa,⁸⁵ clear and dark chief cells. This variation suggests that the cell types are not clearly differentiated and that transition forms are numerous.

In 1903 Erdheim⁵⁰ showed the presence of fat granules in the chief cells as well as in the stroma. Very little was seen in the oxyphil cells. The fat was not present at birth and began to appear after the third decade, gradually increasing with age. He emphasized the fact that the fat content was not dependent upon the nutrition of the individual, but only upon age.

Kurokawa⁸⁵ studied a larger series of about 815 glands removed from 240 autopsy cases at Keio University Medical College. His material ranged from a 7 month fetus to individuals 80 years of age and presents the most adequate survey yet available of the physiological limits of variation. His findings show that up to the age of puberty the cells are all clear chief cells containing glycogen but no fat. At this point these cells begin to decrease and the dark chief and oxyphil cells gradually appear. The dark chief cells contain fat but no glycogen. The oxyphils contain neither fat nor glycogen. Follicles and colloid appear at this time. The oxyphil cells increase with advancing age, occurring in masses and nodules after the age of 30 or 40 years. He found that after puberty there was no tendency for the interstitial connective or fatty tissues to increase, nor was there any atrophy of the gland.

In our study the parathyroid glands were removed from 150 routine autopsied cases. In the majority of these, four glands were found in their normal positions, though in occasional cases only three or even two could be demonstrated. In rare instances supernumerary and aberrant glands have been reported. Parathyroid tissue has been found in the thyroid, thymus, and in other regions of the anterior mediastinum.^{28, 99} The glands in each case are usually the same size, though minor variations are not rare. On the average they measure 3-6 mm. in length, 2-4 mm. in width, and 0.5-2 mm. in thickness. They are usually embedded in fat tissue, from which they can be distinguished by their color, which varies from a dark reddish brown to a light tan.

Our material was for the most part fixed in Zenker's fluid, embedded in paraffin, cut at about 8 microns thickness, and stained with eosin and methylene blue. In numerous instances paraffin sections of formalin-fixed material stained with hematoxylin and eosin were also studied. Variations in fixation and staining technique were often found to alter the appearances of the cells significantly. The fat content was determined from frozen sections fixed in 10 per cent

formalin and stained in scharlach R. Preparations of alcohol-fixed material stained for glycogen with Best's carmine were made in about one-half of the cases. It was found that in all but a few cases the various glands from the same individual were practically identical in appearance. We have elected to recognize four major cell types and have been forced to admit the existence of transition types.

The normal chief or principal cell (Fig. 6) is polyhedral in shape, poorly outlined and measures 6-8 microns in diameter. Its nucleus is large, round, sharply demarcated by a basophilic outline, comprises more than half of the cell volume and measures 4-5 microns in diameter. The chromatin is usually abundant, often giving the nucleus a pyknotic appearance. The cytoplasm is usually very scant and faintly acidophilic. Often it is more or less retracted toward the cell margins, leaving an unstained halo of varying width about the nucleus. This is often spoken of as vacuolization, though it may represent merely an artefact of fixation and dehydration. Formalin fixation tends to exaggerate this appearance, and in frozen sections of unfixed tissue vacuolization is difficult to demonstrate even when paraffin sections show it in marked form. Cells showing this halo formation in moderate degree we have termed transition wasserhelle cells.

When the cytoplasm is apparently entirely absent, complete vacuolization, the cell is called a "water-clear" or "wasserhelle" cell. At this stage the cell is sharply outlined and is larger than the chief cell, measuring 10-15 microns in diameter. Its nucleus is about the same size as that of the chief cell, but is usually more hyperchromatic, more often pyknotic, and eccentrically located. These cells are seen only occasionally in the apparently normal gland and then in small groups. We have not observed them before puberty. Their presence in small clusters has sometimes been interpreted as focal hyperplasia. When the whole gland is composed of these cells, as in some cases of nephritis and hypertension, it is felt that hyperplasia is definite.

The pale oxyphil cell (Fig. 7) is polyhedral in shape, has a sharply demarcated cell margin and measures 11-14 microns in diameter. The nucleus is also about the same size as that of the chief cell, but not so hyperchromatic. The cytoplasm is uniformly reddish pink, finely granular and completely fills the cell. There is no vacuolization.

The dark oxyphil cell is larger than the chief cell but smaller than the pale oxyphil, and measures 8–10 microns in diameter. Its cell border is not sharp. The nucleus is small, 3–4 microns, and intensely pyknotic. The cytoplasm is dark red and homogeneous.

The distribution of these cells varies with age. Until puberty the gland is composed wholly of chief cells with a slight tendency to vacuolization (Fig. 1). We cannot subscribe to Kurokawa's⁸⁵ classification of them as wasserhelle cells. These cells contain a fair amount of glycogen but no fat, the latter appearing soon after puberty as very fine droplets. At puberty or soon afterward pale oxyphils gradually appear, at first singly and then in pairs. They increase in number with advancing age, forming large islands usually after 40 to 50 years of age (Fig. 3). These islands are sharply circumscribed but not encapsulated, and often continuous cords of parenchymal cells can be traced across the margin into the surrounding tissue. These cells do not contain fat or glycogen. Dark oxyphil cells occur singly and usually close to the stroma. They are not present before puberty, and occur usually when pale oxyphils are present. They likewise do not contain fat or glycogen.

Following puberty large fat cells appear in the stroma and increase in number until about 40 years of age (Fig. 2). The fat tissue remains fairly constant during middle age and does not increase with old age. In fact, in cases where the individual was over 80 years of age, in which oxyphil groups are numerous, it is somewhat diminished. It is interesting to note that when an adult gland is smaller than normal the decreased size is due to the absence or marked diminution of fat cells, whereas the parenchymal cell volume is about the same as in a normal sized fat-containing gland. These observations on the fat content do not wholly coincide with those of Erdheim.⁵⁰

Cysts of varying sizes are observed in about one-half of the cases beyond puberty (Fig. 4). In 2 cases one of the glands was composed almost wholly of one large cyst similar to that described by Alagna.² These cysts are filled with granular and cellular débris or with a dark blue-staining, finely granular material which is often, though perhaps improperly, termed colloid.

In one case a small, circumscribed, apparently non-functioning adenoma 3–4 mm. in diameter with a distinct fibrous capsule was found in one of the glands. The cells of this tumor were classified as a transition stage between chief and pale oxyphil cells (Fig. 5).

CASES OF HYPERPARATHYROIDISM

The clinical and surgical aspects of many of these cases have already been reported elsewhere in detail and reference to them will be given with each description. Cases 1-17 are collected in a paper by Albright, Aub and Bauer.⁴ The surgical features in the treatment of these cases have been reported by Churchill and Cope.^{32, 33} The case numbers in the present series have been kept identical with those of the clinical studies.

With the gradual accumulation of material it became evident that one fundamental line of division could be drawn. One group of cases, the smaller one, was characterized by a diffuse, uniform alteration of all the parathyroid tissue in the body (Figs. 8-13). In the second group one gland, often only a part of it, rarely parts of two glands, were abnormal (Figs. 15-26), whereas the remaining parathyroid tissue was grossly and microscopically within the limits of normal variation. That the first group is to be regarded as hyperplastic, dependent on some external chemical, hormonal or nervous stimulation, seems obvious. That the second group falls within the accepted limits of neoplasia is a thesis we shall attempt to defend. The case reports which follow have been grouped according to this general classification and then further subdivided on purely morphological grounds of similarity of cell type and structure.

GROUP A: HYPERPLASIA

CASE 15^{8, 4, 32} (33-4840 and 34-796). *Clinical History:* A. P., a widow, 62 years of age, was first admitted in 1928 because of bilateral renal calculi. At this time two stones were removed from the right kidney pelvis, but in November, 1933, bilateral renal calculi were again found. The non-protein nitrogen was 32 mg. and the phenolsulphonephthalein excretion was only slightly impaired. The urine contained many white blood cells. A stone was removed from the left ureter. Serum calcium was 15 mg., phosphorus 2.2 mg., phosphatase 7.3 Bodansky units. X-rays of the skeleton were negative. On Dec. 19, 1933, at operation a parathyroid tumor was demonstrated below the right lobe of the thyroid and another on the left in a symmetrical position. Both were removed and the dissection was carried no further. Postoperatively the serum calcium at first fell, but soon began to rise. On Dec. 26, 1933, the serum calcium was 15.4 mg., the phosphorus 2.2 mg. On Feb. 28, 1934, a second operation was performed at which the left upper parathyroid was not demonstrated, but the right upper was found greatly enlarged and was resected, a portion about twice the size of a normal gland being left in place. In April, 1934, the serum calcium was 11.4 mg., the phosphorus 2.2 mg. When last seen, on June 30, 1934, the serum calcium was 13.78 mg., the phosphorus 2.75 mg.

Gross Description: The specimen removed from the region of the lower pole of the right lobe of the thyroid weighs 0.61 gm. and measures 1.5 by 1.2 by 0.5 cm. The gland is encapsulated, smooth surfaced and deep reddish brown in color. On one surface there is a curved shallow depression. The cut surface is homogeneously reddish brown. The specimen removed from the region of the lower pole of the left lobe of the thyroid weighs 0.51 gm. and measures 1.8 by 0.8 by 0.6 cm. It is slightly paler but otherwise the same as the other specimen.

The specimen removed 71 days later (34-786) from the region of the upper pole of the right lobe of the thyroid weighs approximately 10 gm. and measures 5 by 3 by 1.3 cm. Its surface is reddish brown, smooth and glistening, except where several small clear cysts averaging 0.5 cm. in diameter project from it. On section the surfaces are reddish brown, with cysts which yield a little clear straw-colored fluid.

Microscopic Examination: Both specimens removed at the first operation have the same microscopic appearance. The capsule is thin and no normal parathyroid tissue is found within or outside of it.

There is only one type of cell throughout, the wasserhelle cell (Fig. 9), which is polyhedral in shape, sharply demarcated by a thin eosinophilic membrane, and varies from 10 to 40 microns in diameter, averaging 15 to 20. Many of the cell boundaries are broken, with resultant fusion, similar to the fusion of alveoli in pulmonary emphysema. In contrast to the variability in the size of the cells the nuclei, though often multiple, are all approximately the same size, averaging about 8 microns in diameter. They are round to slightly ovoid in shape, sharply outlined, moderately hyperchromatic, with an eccentrically placed nucleolus. As a rule the nuclei are located in the end of the cell that is contiguous to the stroma. This produces a characteristic pattern which resembles branches of berries (Fig. 11). The cytoplasm is clear except for a little, light pink-staining granular material. Many of these tiny granules are glycogen deposits. Similar granules are present within the nuclei. There is no fat, except for a rare droplet in the stroma. The low power appearance of the histological sections is so similar to that of clear cell renal carcinomas that distinction would be difficult if the source were not known. In fact one gland of this type was actually reported as a hypernephroma of the thyroid.⁸⁴

The stroma is composed of thin, fibrous connective tissue bands containing a moderate number of connective tissue cells and relatively few blood vessels. These bands surround small and large groups of cells, producing a pseudoglandular effect. This effect is further emphasized by the position of the nuclei, as mentioned above. Occasionally a true single layered alveolus is seen. No oxyphil or chief cells are found. There are no mitoses.

The cells in the specimen removed at the second operation are of the same type as those present in the other two glands. The same characteristic pattern is produced by the peripheral location of the nuclei, but there is more marked gland and cyst formation. These spaces vary in size from 0.1 to 5 mm. They are usually lined with a single layer of wasserhelle cells. Their lumina are filled with pink-staining granular debris and at times with desquamated lining cells and red blood cells (Figs. 8 and 9).

CASE 16^{8, 4, 32} (34-634). *Clinical History:* T. F., a male, 26 years of age, entered on the urological service because of intermittent attacks of right renal colic for 15 months. Physical examination was negative. The non-protein nitrogen was normal. Serum calcium was 15.1 mg., phosphorus 1.8 mg. The urine showed finely granular casts of the hyperparathyroidism type (containing calcium phosphate).⁷ X-rays of the skeleton were negative, but films of the urinary tract showed two small stones in the right ureter. These were removed and later, on Feb. 16, 1934, a parathyroid tumor below the right lower pole of the thyroid at the sternoclavicular junction was resected. Directly beneath this lay a second, much larger tumor, which came from the surface of the prevertebral fascia and the posterolateral aspect of the trachea and esophagus. This was also excised. The following day the serum calcium was 11.9, the phosphorus 2.6 mg. When last seen, on April 13, 1934, the serum calcium was 10.2, the phosphorus 2.3 mg.

Gross Description: The first gland is a well circumscribed, encapsulated, smooth surfaced, orange-brown ovoid mass weighing approximately 0.6 gm. and measuring 1.5 by 1 by 0.6 cm. The cut surface is uniformly yellowish brown.

The second gland is an encapsulated, smooth surfaced, ovoid soft tumor weighing approximately 15 gm. and measuring roughly 4.5 by 3.5 by 2.5 cm. Both poles are slightly pointed and narrowed. The surface is reddish brown. At one pole there is a small cyst 0.8 cm. in diameter filled with clear colorless fluid. The cut surface is homogeneously yellowish to reddish brown, soft and glistening.

Microscopic Examination: The microscopic picture of both of these tumors is identical. They are completely made up of large wasser-

helle cells of the same type as that seen in Case 15. There are no chief or oxyphil cells. No normal parathyroid tissue is present.

The cells in this case, as in Case 15, are in many places arranged in true gland formation. The characteristic pattern produced by the peripherally placed nuclei is much more pronounced than in Case 15 (Figs. 10 and 11).

CASE 17^{8, 4, 32} (34-600). *Clinical History:* J. M. M., a female, 55 years of age, entered on the urological service for intermittent attacks of renal colic for the past 14 months. Physical examination was negative. A stone demonstrated by X-ray in the right ureter was removed. The non-protein nitrogen was 31 mg. Serum calcium was 12.7 mg., phosphorus 2.4 mg., phosphatase 4.2 Bodansky units (normal). X-rays of the skeleton were negative. At operation on Feb. 14, 1934, all four glands were found in normal position and were enlarged. Three, and a portion of the fourth were removed. The following day the serum calcium was 10.9 mg., the phosphorus 2.2 mg. When last seen, on June 25, 1934, the serum calcium was 10.34 mg., the phosphorus 2.99 mg.

Gross Description: (Left Upper): A soft, reddish brown, slightly nodular, irregular, non-granular piece of tissue weighing 2 gm. and measuring approximately 2 by 2 by 1 cm. The specimen has been cut in several places. (At operation the recurrent laryngeal nerve had to be dissected free.)

(Left Lower): Weighs 0.6 gm., measures 1 by 0.8 by 0.4 cm.

(Right Upper): A small biopsy approximately 1 mm. in diameter.

(Right Lower): Weighs 0.8 gm., measures 1.8 by 1.2 by 0.4 cm.

The cut surface of all the specimens is homogeneously reddish brown.

Microscopic Examination: The microscopic picture of all sections is identical with Cases 15 and 16.

CASE 23 (34-2729). *Clinical History:* A. S., a male, 41 years of age. In August, 1932, the patient passed a renal stone. Three months later his physician removed three stones with the aid of a cystoscope. One month later he complained of a tight feeling in the left hip, soon followed by soreness in the hypogastrium and left lower quadrant. Cystoscopy was negative. He lost 35 pounds in weight. At the Massachusetts General Hospital in Feb., 1934, X-rays showed a cystic tumor of the left ilium and calcification in the lower pole of the left kidney. The bone lesion was resected and was diagnosed as an atypical chondrosarcoma. No changes in the least suggestive of osteitis fibrosa cystica were demonstrated. Examination of the urine showed a slight trace of albumin, 20-60 white blood cells, and occasional red blood cells. Bence-Jones protein was found on one occasion. The serum calcium was 13.1 mg., serum phosphorus 2.92 mg. After discharge the patient felt somewhat better for a while but soon the abdominal symptoms returned and he reentered for parathyroidectomy. The laboratory findings were essentially the same. On July 11, 1934, operation was performed.

The left upper, left lower, and right lower parathyroid glands were found enlarged and removed. A small biopsy was taken from an apparently normal sized right upper. Postoperatively the serum calcium went down slowly, reaching 10.18 mg. on the 10th day; the phosphorus was 3.4 mg. Three months later the corresponding figures were 11.96 mg. and 3.69 mg.

Gross Description: (Right Lower): a reddish brown, slightly flattened, smooth surfaced soft mass weighing 0.13 gm. and measuring 8 by 6 by 3 mm.

(Right Upper): A small biopsy 1 mm. in diameter.

(Left Upper): A multilobulated, reddish to yellowish brown, irregularly shaped, smooth surfaced mass weighing 2.18 gm. and measuring approximately 3 by 1.7 by 0.8 cm. The largest lobule measures 3 by 1 by 0.8 cm. and is more yellow than the rest of the specimen.

(Left Lower): A deep reddish brown, ovoid soft mass weighing 0.16 gm. and measuring 1.1 by 0.6 by 0.3 cm. The cut surfaces of all the specimens are homogeneously reddish brown.

Microscopic Examination: The cells are all of the large wasserhelle type, arranged in gland formation and showing the typical pattern seen in Cases 15, 16 and 17.

CASE 25 (34-4318). *Clinical History:* W. P., a male, 39 years of age, began in August, 1934, to have attacks of sharp pain in the right flank. The pain occurred suddenly at any time of the day or night and lasted 2 to 3 minutes. On October 1st he was awakened at night by an attack which persisted for several hours and which was finally relieved by morphia. During a 3 day period of observation at a local hospital he had three more similar attacks, all requiring morphia for relief. On Oct. 4, 1934, he was admitted to the Massachusetts General Hospital where a stone, demonstrated by a pyelogram, was removed from the right ureter. X-rays of the skeleton were negative. The serum calcium was 13.91 mg. per 100 cc., serum phosphorus 2.96, and phosphatase 3.67 units. At operation on October 27th the right upper parathyroid was found enlarged. A rush frozen section of a biopsy of this gland showed large wasserhelle cells typical of hyperplasia.* With this information the surgeon continued his search for the other glands and exposed all four. Both uppers were markedly enlarged and were resected. The lowers were much smaller. One of them was completely removed and three-quarters of the other was resected. On October 29th the serum calcium was 9.43 mg. per cent, serum phosphorus 1.34. There were no signs of tetany. The patient was discharged on Nov. 5, 1934.

Gross Description: (Right Upper): An irregular, slightly lobulated tumor weighing 4.96 gm. and measuring approximately 3.7 by 2 by

* In order to bring out the marked vacuolization of these wasserhelle cells it is advisable before freezing to fix the tissue by heating it to the boiling point in 10 per cent formalin.

1 cm. At one pole there is a long pseudopod-like projection 5 mm. in length and 3 mm. in diameter.

(*Left Upper*): Weighs 1.63 gm. and measures 1.7 by 1.7 by 0.6 cm. with a pseudopod-like tab 5 mm. in diameter.

(*Left Lower*): Weighs 0.11 gm. and measures roughly 4 mm. in diameter.

(*Right Lower*): Two small pieces weighing 0.10 gm.

The surfaces of all the specimens are smooth and reddish to yellowish brown. The cut surfaces are homogeneously pink to yellowish gray, moist and translucent.

Microscopic Examination: The microscopic picture of all sections is identical with Cases 15, 16, 17 and 23. A piece of thyroid removed at operation was histologically normal.

Summary of Cases 15, 16, 17, 23 and 25 (Wassershelle, Generalized)

The similarity of these 5 cases is at once apparent from an examination of the sections. The uniform, unusually large clear cells, the tendency to acinar arrangement and the basal orientation of the nuclei present a uniformity of appearance that is entirely lacking in the group of localized tumors to be described below. It differs also from the hyperplasia produced experimentally in rabbits by the injection of an extract of the anterior pituitary. In these experiments Hertz and Kranes⁷⁴ found enlarged chief cells with comparatively slight vacuolization and numerous mitoses.

The similarity to the clear cell renal adenocarcinoma is striking and in fact, as already reported in the text, has misled previous observers.⁸⁴

In Cases 17, 23 and 25, histological specimens were obtained from all four glands and diffuse involvement of all the parathyroid tissue proved beyond doubt. In Case 15 only three glands could be demonstrated in two extremely thorough operative dissections. In Case 16 only two enlarged glands were demonstrated, but no search was made for the other glands at the time of operation and the patient has not returned to the clinic. However, the histological similarity to the other cases makes us feel that it should be classified with this group.

CASE 23A* (7119). *Clinical History:* R. N., a female, 25 years of age, entered the Maine General Hospital in April, 1933, complaining of weakness and fatigue. She had had polyuria and polydipsia all her life, with no recent change. Her teeth had all been loose for 7 years. She had had pain, weakness and numbness of the legs for the past 8 months. Examination showed a small mass 8 by 4 mm. in the middle of the right neck. The blood pressure was 158/78. Examination of the urine showed a fixed specific gravity around 1.005, 40-60 mg. of albumin, and 10-20 white blood cells. Examination of the blood showed a red cell count of 1,980,000, with a hemoglobin of 35 per cent. The white blood cell count was normal. The non-protein nitrogen of the blood was 150 mg. On her second admission in September, 1933, the laboratory examinations were about the same. X-rays of the skull, ribs and hands showed definite cysts, as well as calcification of the arteries and subcutaneous tissues. The serum calcium was 8 mg., serum phosphorus 9 mg. Although these blood studies appear paradoxical, they may be explained according to Albright by the marked renal damage. The phosphate retention secondarily causes a diminution in blood calcium. The patient died Nov. 6, 1933. Postmortem examination showed in addition to the parathyroid enlargement a marked chronic pyelonephritis and osteitis fibrosa. A clinical diagnosis of renal rickets could not be ruled out.

Gross Description: All the parathyroid glands except the right lower are enlarged. The left lower is ovoid in shape and measures 9 by 7 by 3 mm. The left upper is the largest and measures 17 by 8 by 5 mm. It contains on its posterior surface a circular nodular elevation 3 mm. in diameter. The right upper measures 10 by 8 by 4 mm. The glands are smooth surfaced, and in spite of having been previously fixed in formalin still retain a slight yellowish brown tint.

Microscopic Examination: All the glands, including the normal sized one, show essentially the same process in varying degrees, the most marked changes being in the largest gland. The capsules are thin and not remarkable. The predominating cell is the chief cell, which is normal in size, averaging 8 microns in diameter. The cell outline is poorly visualized. The nucleus is round, deeply pyknotic and hyperchromatic, sharply outlined, measures 5-6 microns in diameter, and fills more than two-thirds of the cell volume. The cytoplasm is acidophilic and coarsely granular. The cells themselves are, therefore, not hypertrophied.

The arrangement of the cells presents the most significant picture.

* In addition to the 24 surgical cases included in this study, we have had the opportunity to study the parathyroid glands removed at autopsy from a case at the Maine General Hospital. The findings are quite different from any of our own cases. The authors wish to thank Drs. John Hamel and Mortimer Warren for permission to report the pathology of this case.

In order not to break the series of cases from the Massachusetts General Hospital, this case is numbered 23A instead of 24.

Small groups of these cells, 10-20 in number, are surrounded by a thin connective tissue band often containing a very small capillary. The nuclei tend to be located at the bases of the cells and produce a definite pseudoglandular arrangement, although there is no lumen. Each group appears to be shrunken away from its connective tissue band, leaving a clear space about 6-9 microns in width. In many places, especially in the larger glands, acinar arrangement is much more marked, forming well circumscribed, papillary foci measuring up to 0.8 mm. in diameter (Figs. 12 and 13). These resemble closely the basophilism frequently observed in the pituitary. There is very little inter- or intracellular fat tissue.

Scattered throughout all sections, but more marked in the larger glands, are groups of typical, normal sized, pale oxyphil cells arranged in the same formation as the chief cells, even including the papillary form. There are no wasserhelle cells.

Summary of Case 23A (Hyperplasia, Chief Cell Type)

This patient showed three enlarged and one normal sized parathyroid gland all made up of normal sized chief and pale oxyphil cells arranged in pseudoglandular and papillary formations. There is very little fat tissue and no wasserhelle cells.

GROUP B: NEOPLASIA

CASE 6^{118, 68, 16, 97, 6, 5} (32-3985). *Clinical History:* C. M., a male, 35 years of age. The clinical history of Captain Martel has appeared so often in the literature that no attempt will be made to repeat it. The diagnosis made by Dr. Eugene DuBois in 1926 was the first clinical recognition of hyperparathyroidism in this country, and probably the second in the world. The coöperative attitude of the Captain made possible a series of metabolic studies that are unparalleled and have contributed enormously to our knowledge of the disease.

Operation was performed first by Dr. E. P. Richardson in 1926, and two normal parathyroid bodies were removed. An article describing the case has been widely misquoted as recording improvement following this operation, but any such tendency was temporary and may more fairly be attributed to the high calcium diet. As the disease progressed the renal damage became more marked and stones formed in the kidney pelvis. The serum calcium averaged 15 mg., phosphorus 2.3 mg. At the seventh operation, by splitting the sternum a parathyroid tumor was found in the anterior mediastinum. Subtotal resection was performed. Severe tetany supervened, which was controlled with difficulty, owing to acidosis attributable to the renal damage. Six weeks after the removal of the parathyroid tumor a stone was passed into the left ureter, causing complete obstruction. With the patient in a dangerous balance between tetany and aci-

dosis and in the face of a markedly diminished renal function, a left ureterolithotomy was undertaken. Death occurred 26 hours later. Postmortem examination showed well marked osteitis fibrosa cystica with early evidence of healing, and marked renal calcinosis.

Gross Description: A smooth, round, hard nodule 2.5 cm. in diameter. On cutting through the nodule it is seen to have a calcified shell 1-2 mm. in thickness, the rest of the tumor being made up of soft, shiny, brownish material. The tumor has a soft fibrous pedicle.

Microscopic Examination: The tumor is made up of only one type of cell, the chief cell (Fig. 17), which measures about 8-11 microns and has a poorly demarcated, pinkish, polyhedral cell outline. The nucleus is large, filling about one-half of the cell body, is round, sharply demarcated, and contains a variable amount of chromatin. Many of the nuclei, especially of the smaller cells, are deeply basophilic and almost pyknotic. The cytoplasm is light pink, coarsely granular and in many places reticular. A large proportion of the cells has a vacuolated halo around the nucleus and in a few the vacuolation extends to the periphery. An occasional cell is multinucleated; there are no mitoses. The cells contain no fat. There are no typical wasserhelle or pale oxyphil cells and only an occasional dark oxyphil cell.

The cells are arranged in compact masses, columns, and in pseudoglands, though one section shows a few definite acini lined with chief cells and filled with pink-staining, homogeneous material. The vessels of the intervening stroma are more numerous, much larger and much more congested than those in the normal parathyroid. Scattered throughout the stroma are variable sized spaces without demonstrable lining measuring up to 1 mm. in diameter. Many of these are empty, but others contain colloid-like, pink-staining material or red blood cells, and occasionally a desquamated parathyroid cell. There are also small colloid droplets throughout the stroma.

CASE 7 (32-4132). *Clinical History:* M. R., a female, 36 years of age. In 1929 the patient developed dull pain in her arms and legs. In 1931, at another hospital where her bones were found to be decalcified and filled with cysts, a diagnosis of osteitis fibrosa cystica was made. Serum calcium was 14.1 mg., phosphorus 2.9 mg. On Oct. 19, 1931, a tumor, supposedly of parathyroid origin, was excised, but microscopic examination revealed thyroid tissue. A biopsy of the mandible, 6 months later, showed osteitis fibrosa cystica. On May 6, 1932, a right hemithyroidectomy was done and a tumor at the left lower pole was removed. Microscopic examination revealed thyroid and thymic tissue. In November, 1932, she

entered the Massachusetts General Hospital for the first time. The serum calcium was 12 mg., phosphorus 1.74 mg., phosphatase 16.9 units. X-ray confirmed marked decalcification and multiple fractures in the pubic bones. She had paralysis of the right vocal cord. On Nov. 15, 1932, after fruitless exploration of the neck, a tumor was found in the anterior mediastinum beneath the costal cartilage of the second rib at the border of the sternum. A subtotal resection was done. During convalescence the patient developed definite tetany, associated with a fall in serum calcium. On Nov. 29, 1932, the serum calcium was 5.16 mg., phosphorus 3.29 mg. When last seen, on May 17, 1934, the bones showed great improvement. The serum calcium was 8.39 mg., phosphorus 3.03 mg. The phosphatase was 5.08 units.

Gross Description: A pedunculated, brownish, lobulated, firm encapsulated tumor measuring 2.5 by 3 by 1 cm. The cut surface is brown and shows many firm lobules varying in size from 3–6 mm. in diameter.

Microscopic Examination: About the same as in the preceding case. Palisading of cells is very prominent. In many places the columns are so winding and the intervening vascular stroma so abundant that there is almost a papillary arrangement. Scattered throughout the stroma are numerous large mast cells.

CASE 9 (33-1226). *Clinical History:* J. R. C., a male, 33 years of age. In 1931 the patient gradually developed weakness, loss of weight, nocturia and pains in the legs noticeable when walking. In 1932 the pain in the legs was almost constant when upright. He became much weaker, lost 30 pounds in weight, and by January, 1933, was unable to work. A diagnosis of hyperparathyroidism was made at the Boston Dispensary, where X-rays were taken of his bones and calcification of the kidneys observed. A history of mild polyuria and polydipsia was elicited. In March, 1933, he entered the Massachusetts General Hospital, where X-rays showed marked generalized decalcification of the skeleton with cyst formation. There was a markedly depressed renal function, a low urine specific gravity and a secondary anemia. The serum calcium was 16.9 mg., serum phosphorus 3.02 mg. On April 4, 1933, at operation a tumor lateral and posterior to the lower portion of the left lobe of the thyroid was resected. The dissection was limited to the left side of the neck. Convalescence was characterized by prolonged and severe tetany. When last seen, on July 24, 1933, steady improvement in appetite and strength was noted. He had gained 30 pounds in weight, had much less pain on walking and less nocturia. The anemia had improved moderately although the renal function was the same. Calcium and phosphorus values were normal, but phosphatase was slightly elevated. X-rays showed no change in skeleton or kidneys.

Gross Description: An ovoid, yellowish pink, soft, encapsulated mass measuring 4 by 2.2 by 2 cm. and weighing 7 gm. The cut surface is soft, friable, mushy and yellowish gray.

Microscopic Examination: The capsule is slightly thick, in places

measuring almost 1 mm. It is composed of fibrous connective tissue with only a few cellular areas. One of these areas is close to the inner surface of the capsule and contains lymphocytes, red cells and deposits of hemosiderin.

The cells in this case are larger than those of the preceding 2 cases, measuring 11 to 14 microns in diameter. The nuclei measure 8-10 microns and are hyperchromatic, but no definite multinucleated cells or mitotic figures are seen.

Summary of Cases 6, 7 and 9 (Chief Cell Type Alone)

These 3 cases are all composed of large, hyperchromatic chief cells arranged in pseudoglandular and columnar formation (Fig. 17). Cases 7 and 9 show well marked palisading. Neither the cells nor the stroma, which is vascular, contain any fat. There are no wasserhelle or pale oxyphil cells, and only an occasional dark oxyphil cell. Multinucleated cells are rare and no mitoses can be found.

CASE 1 (30-3056). *Clinical History:* M. J. S., a female, 46 years of age. In 1916, following a miscarriage, the patient noted a swelling in the right side of her neck. Between 1928 and 1930 she developed pain successively in the right arm, right thigh, left thigh and right forearm. A biopsy of the right ulna at the Worcester Memorial Hospital showed a giant cell tumor, for which she was given X-ray therapy. Numbness and pain in the legs increased. She entered the Massachusetts General Hospital where X-rays showed generalized osteitis fibrosa cystica. A secondary anemia was present. Serum calcium was 13.68 mg., phosphorus 2.58 mg. At operation a tumor was seen pushing the right lobe of the thyroid forward. Hemithyroidectomy with total resection of the tumor was performed. After 2 months the pains had diminished and appetite, strength and gait had improved, though anemia was still present. Calcium and phosphorus levels were normal. By the 8th postoperative month she had gained 40 pounds, all skeletal pain had ceased and constipation had disappeared. Her strength was better than since 1916. X-rays showed slight but definite improvement in the bones. Two and a half years later she felt very well except for occasional backache. The cysts remained unchanged by X-ray. The serum calcium was 9.38 mg., phosphorus 3.41 mg., phosphatase 2.58 units.

Gross Description: A light brown, ovoid, moderately firm, encapsulated mass, measuring 6.5 by 5 by 3.5 cm., and weighing 53.2 gm. The cut surface is homogeneously pale, glistening and yellow brown.

Microscopic Examination: The major portion of all sections is made up of closely packed cells arranged in pseudoglandular, cord or strand-like columnar formations. The pseudoglandular areas are composed of irregular, rounded groups of cells, from five to fifty in each group. The columnar areas are usually two or three cells in

width, but are comparatively few in number as compared with the pseudo-alveolar.

The predominant cell is polyhedral in shape and varies in size from 5 to 20 microns (Fig. 18). Its cell outline is often indistinct, but in many places can be made out as a thin, slightly irregular pink line. The smaller cells have round and light staining nuclei; the larger, some of which are almost 20 microns in diameter, have large, irregular, and more hyperchromatic nuclei, sometimes reaching the size of four to six ordinary nuclei. Chromatin is abundant and usually a nucleolus can definitely be made out. For the most part the cytoplasm immediately surrounding the nucleus is absent, producing a halo from the periphery of which pinkish granules extend to the cell margins, though a fair proportion of the cells have a pink, finely granular cytoplasm which completely fills them. A small proportion of these cells has one or two small fat granules in its cytoplasm, but the majority of them contain no fat. Multinucleated cells are fairly numerous; some contain as many as seven nuclei. Although no mitoses are seen, the atypicality of the nuclei, as evidenced by the variation in their hyperchromatism and the frequency of multinucleated cells, strongly suggests a neoplastic rather than a hyperplastic process.

Single, dark oxyphil cells are present in small numbers. They are of normal size, about 8-10 microns in diameter, with a dark, deeply basophilic irregular nucleus surrounded by a deeply eosinophilic, finely granular cytoplasm. The nuclei are either small, about one-fifth the cell volume, or large, about three-fourths of the cell volume. These cells lie for the most part close to the interstitial stroma and are not found in groups. No wasserhelle cells are seen.

The stroma is composed of thin fibrous strands which pass around groups of cells, producing pseudo-alveoli or columns. A small proportion of the stroma is acellular, but most of it contains thin-walled capillaries lined by typical endothelial cells. In some areas larger vessels partially filled with blood are seen. There are practically no fat cells in the stroma.

Scattered throughout the tumor are irregularly shaped spaces varying in size from 50 to 200 microns. They have a connective tissue lining, are surrounded by masses of chief cells, and contain for the most part pink-staining, granular debris, occasionally a few red blood cells, but no colloid. Besides these there are also much larger,

irregular confluent spaces without any definite lining, which also contain pink-staining granular débris. These appear to be areas of localized edema of the stroma.

CASE 11 (33-2429 and 33-4429). *Clinical History:* M. T., a female, 53 years of age. From 1920 to 1923 she had pain in the legs and disturbance in gait. In 1929 an operation was performed on a giant cell tumor of the upper jaw. Later a second tumor in the nose was curetted. In 1930 she fractured the left forearm and left tibia by tripping on a rug. Abdominal pain led to an operation for replacement of the uterus. The severe pains in the legs continued and in 1931 a cystic tumor of the right tibia, discovered by X-ray, was curetted, but she remained completely invalided except for limited activity with crutches. In 1933 severe pain in the legs and lower back was associated with a spontaneous fracture of the neck of the right femur and a diagnosis of osteitis fibrosa cystica was made at the Cambridge City Hospital. She was transferred to the Huntington Hospital where the diagnosis was confirmed by Dr. J. C. Aub. X-rays showed extensive decalcification and cyst formation of the entire skeleton and calcium deposits in the kidney parenchyma. Her condition seemed critical, with respiratory distress, nausea and vomiting. The serum calcium was 13.7 mg., serum phosphorus 2.4 mg., the phosphatase elevated. Renal function was diminished. At operation at the Massachusetts General Hospital on June 24, 1933, a subtotal resection of parathyroid tumor found behind the left upper pole was performed. Approximately 1 gm. of parathyroid tissue was left. A nodular goiter was noted. A mild tetany developed, the calcium falling as low as 7.4 mg. A second fracture of the femur occurred when the traction apparatus was changed. In September, 1933, she was comfortable but the bones showed no evidence of increased density and the fractures failed to develop callus. The extreme decalcification of the skeleton was treated with a high calcium diet, vitamin C, viosterol and calcium glycerophosphate. The serum calcium rose to 11.53, the phosphorus was 3 mg. On Nov. 15, 1933, a second operation was done, with excision of the remainder of the parathyroid tumor. One week later the calcium was 8.47 mg., the phosphorus 3.12 mg. When last seen, on March 17, 1934, locomotion was much improved, and the fracture seemed solid.

Gross Description: A partially encapsulated, smooth brown nodule measuring 3 by 2 by 1 cm. and weighing 3.85 gm. Many vesicles are present on the surface. On section the center of the tumor is found to be composed of a calcified area roughly 5 mm. in diameter. The tumor tissue is homogeneously brown and firm.

A second piece of similar tissue measuring 2.1 by 1.1 by 0.8 cm. and weighing 1.2 gm. is also present.

Microscopic Examination: The capsule is moderately thickened and composed predominantly of acellular fibrous connective tissue. In a few areas there are some connective tissue cells and clumps of lymphocytes. The subcapsular vessels are markedly distended and congested.

This tumor contains three types of cells. The one that makes up the bulk of the tumor is the enlarged chief cell, similar to that seen in the group just described (Cases 6, 7 and 9). In sharp contrast to these cells there are intermingled with them large numbers of cells apparently in the same class as the chief cell, but tremendously enlarged, much more hyperchromatic, and similar to the giant type of cell described in the previous case. Under low power each field appears to be irregularly studded with them. These cells lie side by side and make up about one-third of the whole mass. They vary greatly in size, the smaller ones averaging 14-17 microns in diameter, the larger about 20 microns; many reach as high as 30 microns (Fig. 18).

The third type of cell in this specimen is similar to the slightly enlarged chief cell, except that its cytoplasm is homogeneously much more eosinophilic. These cells are arranged in large clumps and located only at the periphery. They are not the characteristic pale oxyphil cells but are merely a slight variation from the normal chief cell. No true pale oxyphil cells are seen.

The whole tumor has a well marked pseudoglandular arrangement. Although most of this formation is produced by columns and masses of cells surrounding and bordering capillaries and larger blood vessels, a large proportion is formed around unclassifiable spaces, many of which are filled with granular, blue-staining débris. In many places the cells bordering these spaces or blood vessels show definite palisading. Here the nuclei are located at the pole of the cell, away from the apparent glandular lumen. In places where only a single layer of cells surrounds a small capillary the nuclei are also at the pole of the cell, more distant from the capillary lumen. All types of cells share in this arrangement, although there is a tendency for the smaller ones to border the pseudogland or space.

The stroma consists of scanty strands of fibrous connective tissue, a small amount of fat, and vessels which are increased in number but not remarkably congested. No colloid is seen.

The remainder of the tumor (33-4429), removed at the second operation, is an irregular, orange-gray piece of tissue measuring 6 by 4 by 3 mm. Microscopically it is similar to the specimen removed at the subtotal resection. There is a large amount of scar tissue containing many foreign body giant cells distributed around and throughout the specimen, undoubtedly due to the trauma of the previous operation. The same three types of cells are found. The only

possible difference between the two specimens is the relatively increased vesiculation of the chief cells. The latter, however, have not reached the stage in which they can be called true wasserhelle cells.

An interesting incidental finding was the removal of a small piece of pinkish gray tissue measuring 1.5 by 0.7 by 0.4 cm., containing a small cyst-like structure 4-5 cm. in diameter. At operation this specimen, which was in the same field, was believed to be parathyroid. Microscopically it is a papillary cystadenoma, possibly carcinoma, probably arising in aberrant thyroid tissue.

Summary of Cases 1 and 11 (Chief Cell Type with Giant Forms)

The 2 cases in this group are similar to those in the first group (Fig. 18). They have in addition large numbers of giant forms of exceptionally hyperchromatic chief cells measuring up to 30 microns in diameter, many of which are multinucleated. There are no wasserhelle or pale oxyphil cells. The histological picture is strong presumptive evidence for a neoplastic condition.

CASE 3¹ (32-1304). *Clinical History:* C. S., a female, 13 years of age. In 1928 nocturia with enuresis and polyuria developed. She had always drunk large amounts of water. That same year she fractured the right forearm in two places following an injury of considerable violence. In 1930 a limp, at first attributed to fallen arches, was followed in 6 months by the appearance of a deformity of the left knee. This led to the removal of a cyst from the lower end of the femur, and in 1931 a similar deformity of the right knee developed. In March, 1932, X-rays of the skull, pelvis and vertebrae taken at the Massachusetts General Hospital were characteristic of hyperparathyroidism. Almost complete decalcification of the epiphyses, suggesting renal rickets, was interpreted as failure of calcification of growing cartilage. Serum calcium was 12.5 mg., phosphorus 4.7 mg., phosphate 16 Bodansky units. Urinary calcium excretion was within normal limits, total calcium increased. Renal function was greatly reduced and non-protein

Microscopic Examination: The capsule of the tumor is composed of thick fibrous collagenous connective tissue approximately 45 microns in thickness. Just beneath the capsule is a layer six to eight cells deep of normal parathyroid chief cells.

The predominant cell is an enlarged transition wasserhelle cell (Fig. 19). It varies from 10 to 15 microns in diameter, smaller than the typical wasserhelle cell and has a fairly sharply outlined cell membrane. The shape is for the most part polyhedral, although the cells are so closely packed that almost any kind of shape can be found. The nuclei are eccentrically placed, are large, 6-10 microns in diameter, round, with a sharp basophilic outline and filled with a large amount of chromatin. Most of the nuclei contain one large, deeply stained, eccentric nucleolus located close to the limiting membranes. In several there are as many as three definite nucleoli. The cytoplasm is almost completely vacuolated except for some pinkish, finely granular threads on the inner surface of the cell membrane. Fat granules are present in all the parenchymal cells.

Irregularly scattered through the section are focal groups of cells similar to, but distinguishable from, the predominant type. One of these foci, an area 1.5 mm. in diameter, consists of cells with a cytoplasm which is much clearer, cell outlines sharper, and nuclei slightly smaller and more basophilic. The cells in this area might easily be regarded as true water-clear cells. The other focal areas are similar to each other and consist of cells resembling the predominant cell, except that the cytoplasm is stained uniformly light pink. There are several of these groups throughout each section, averaging 0.1-0.15 mm. in size.

A few dark oxyphil cells are scattered singly through the rim of normal tissue and among the vacuolated cells nearby. No oxyphil cells are apparent in the central portion of the tumor. There are a few, very small, colloid-filled vacuoles among the cells just beneath the capsule, but none among the vacuolated cells.

The cells are arranged in pseudoglandular formation, groups of approximately five to twenty-five being closely packed and surrounded by fine connective tissue strands, some of which contain small capillaries. None of these groups of cells shows demonstrable lumina, so that they cannot be called true alveoli. Many of the smaller groups have a single celled layer arranged radially, suggesting rosette formation. The stroma is scant, although there is one

area in which a collection of large fat cells similar to those in the normal parathyroid is seen.

CASE 22 (34-2649). *Clinical History:* H. C. B., a female, 26 years of age, had an attack of left renal colic accompanied by hematuria in June, 1933, followed in the course of a year by three or four similar attacks. There were no other symptoms. She entered the Massachusetts General Hospital in June, 1934, where a left ureterolithotomy was performed. The serum calcium was 12 mg., serum phosphorus 2.65 mg. X-rays of the skeleton were negative. The urine showed a slight trace of albumin and many calcium phosphate casts. A renal function test showed 35 per cent excretion. On July 6, 1934, at operation the right lower parathyroid gland was found enlarged and was removed. A frozen section showed a tumor of the chief cell type. One normal gland was seen in the left upper region. Because of the frozen section diagnosis and the finding of one normal gland, a more extensive exploration was not done. When the patient was discharged the calcium was 10.06 mg., the phosphorus 3.64 mg.

Gross Description: An encapsulated, smooth surfaced, orange-brown soft mass weighing 0.16 gm. and measuring 1 by 0.7 by 0.4 cm.

Microscopic Examination: Around more than one-half of the tumor is a small rim of normal parathyroid tissue. The capsule of the tumor is composed of thick acellular connective tissue measuring approximately 0.5 mm. in thickness. The cells are all of the same type, transitional wasserhelle cells, similar to those seen in Case 3. They average 15-20 microns in diameter with nuclei 10-15 microns in diameter. Many of the cells are much larger and contain as many as seven nuclei. There are occasional small intracellular fat granules. Glycogen is present in all the cells but much more marked in the cells in the rim of normal tissue.

CASE 2 (32-4430). *Clinical History:* A. R., a female, 44 years of age, developed pain in the right hip in 1931. X-rays at the Huntington Hospital in March, 1932, showed an extensive destructive process in the right ilium. There was no appreciable decalcification of the skeleton. The serum calcium was 14 mg., the serum phosphorus 1.5 mg. A diagnosis of hyperparathyroidism was made by Dr. J. C. Aub and treatment with high calcium diet produced improvement of symptoms. X-rays taken at the Massachusetts General Hospital in November, 1932, showed a localized area of rarefaction and areas of increased density were seen in the right ilium and a stone in the right kidney pelvis. On Nov. 30, 1932, a tumor lying against the trachea on the right side posterior to the thyroid was excised. She had very slight postoperative tetany.

Microscopic Examination: The capsule is thin, measuring approximately 30–35 microns, and is composed of acellular fibrous connective tissue. Except for a few places, the immediate subcapsular area is made up of enlarged chief cells, measuring 11–15 microns in diameter. These are usually several layers deep but in some places only a thin rim one to three cells deep.

The arrangement of these cells is variable; some are arranged in small compact masses, some in double or triple rows with a slight tendency to palisading, and others in definite alveolar formation. The connective tissue stroma is very vascular and the capillaries and vessels are congested with polymorphonuclears and red cells.

Toward the central portion of the tumor the cells become more vacuolated, almost reaching the stage of true wasserhelle cells. These transition wasserhelle cells comprise about one-fourth of the total volume.

The most striking feature of this case is the predominance of the oxyphil cells. They are scattered throughout the whole tumor but are more conspicuous near the periphery, occurring for the most part in large groups, although single and small groups of cells are seen everywhere. One group is consistent with the pale oxyphil cell. The other type of eosinophilic cell is more often single, larger, measuring 17–20 microns, polyhedral in shape, has a moderately clear outline and is more deeply stained. The nucleus is small, round, sharply demarcated, hyperchromatic, eccentrically placed and measures about 6–7 microns in diameter. The cytoplasm is deep pinkish red and filled with tiny darker red granules. These cells are similar to those found in the normal parathyroid gland — typical dark oxyphil cells — except that they are much larger. No mitoses are seen.

The stroma, which contains occasional mast cells, is composed of moderately cellular, highly vascular connective tissue, but very little fat. There are occasional collections of lymphocytes and also small, unidentified, pink-staining, granular ovoid masses which do not simulate colloid.

CASE 12 (33–3876). *Clinical History:* Y. D., a female, 51 years of age. In 1925 a left nephrectomy for calculi was performed. She continued to pass gravel intermittently for a number of years. In 1930 a stone was removed from the pelvis of the right kidney. X-ray 1 year later showed a duodenal ulcer. In June, 1933, X-ray showed a stone in the right kidney. The serum calcium was 11.78 mg., phosphorus 4.35 mg., phosphatase normal. A right nephrotomy was performed but she continued to have colicky abdominal pains. A stone was present

in the lower ureter when she was seen 2 months later. There were no bone symptoms. On Oct. 7, 1933, a small parathyroid tumor located just below the sternal notch was removed. A large thyroid adenoma palpated preoperatively was also removed. She developed moderate tetany when the serum calcium dropped on the second postoperative day to 9.03 mg. When last seen, in 1934, the serum calcium was 9.88 mg., phosphorus 3.01 mg. and she felt well.

Gross Description: A small, encapsulated, ovoid mass measuring 1 by 0.4 by 0.2 cm. The surface is pinkish gray and coarsely granular. The cut surface is homogeneously purplish red.

Microscopic Examination: The capsule is very thin and in places not evident. Scattered throughout are large areas of fresh hemorrhage apparently due to operative trauma. At one corner of the section there is a small semilunar-shaped rim of normal parathyroid chief cells. The remainder of the section is made up of the tumor.

The cells comprising the tumor are of two types, the transition chief cell and the wasserhelle cell. The former makes up most of the specimen and shows all gradations from the chief to the wasserhelle cell. The cytoplasm of most of the cells, however, is not so clear as that of the cells of the previous 2 cases (Cases 3 and 22). The true wasserhelle cells, many of which contain no demonstrable nucleus, are compactly grouped in circumscribed islands and are much larger than the transition wasserhelle cells. The tumor resembles certain portions of Case 8, in which foci of wasserhelle cells are prominent, but is more similar to Case 3.

CASE 14 (33-3618). *Clinical History:* M. R., a male, 52 years of age, had an attack of renal colic with slight hematuria in 1923. Similar attacks recurred in 1927 and 1929, and in April, 1933, he developed left costovertebral pain with chills and fever. One month later a left ureterolithotomy was done. The serum calcium was 15.09 mg., phosphorus 2.84 mg. X-rays in November, 1933, showed diffuse skeletal decalcification without cyst formation and small indefinite areas of calcification in the lower poles of both kidneys which were not present in May, 1933. On Nov. 20, 1933, a parathyroid tumor was removed from behind the right lobe of the thyroid just above the inferior thyroid artery. When last seen, on Dec. 4, 1933, the serum calcium was 10.51 mg., phosphorus 2.54 mg.

slightly viscid yellow fluid is withdrawn. The lower half of the tumor is solid reddish brown and smooth surfaced. The cut surface is brownish yellow and moist.

Microscopic Examination: Just beneath the rather thin capsule in one area is a small semilunar rim of normal parathyroid tissue. The remainder of the specimen is composed of transition wasserhelle and true wasserhelle cells. The appearance is similar to Case 12, except that in this case the wasserhelle cells are more numerous than the transition cells.

The large cyst and a few smaller cystic spaces are all lined by wasserhelle cells. Most of them are filled with granular debris; an occasional small one contains colloid.

Summary of Cases 3, 22, 8, 12 and 14 (Transition Wasserhelle, Chief Cell Type)

The cells in these 5 cases are predominantly of the transition wasserhelle cell type — a stage between the chief and wasserhelle cell (Fig. 19). Cases 3, 8 and 22 are closer to the true wasserhelle cell, while Cases 12 and 14 are closer to the chief cell. Small fat granules are present in Cases 3, 8 and 22, but not in 12 and 14. The cells are all closely packed together and have no glandular arrangement. In 4 of the cases there is a rim of normal parathyroid tissue surrounding the tumor. Oxyphil cells are absent in all cases except Case 8. The latter has in addition slightly enlarged chief cells arranged in pseudo-glandular formation.

CASE 10 (33-1943). *Clinical History:* M. S., a female, 54 years of age, developed at the age of 15 years severe "backstrain," which in light of subsequent events may have been a spontaneous fracture of a vertebra. Since then she had experienced frequency and incontinence of urine, culminating in 1932 in severe abdominal pain associated with increased disturbance of urination. There was loss of weight and appetite. Cystoscopy revealed a vesical calculus which was removed by cystotomy. Her renal function was poor. During convalescence routine blood studies showed that the serum calcium was 13.93 mg., serum phosphorus 2.98 mg., and phosphatase 3.4 units. The bones showed some decalcification by X-ray but no cystic areas. Relief from abdominal pain followed the operation, but increasing pain in the thighs made worse by walking led to a second hospital entry for further study of the hyperparathyroidism. On May 24, 1933, at operation a tumor lying adjacent to the left lower pole of the thyroid gland was removed. It was roughly twenty times larger than the normal inferior parathyroid which was seen on the right side. There was no tetany and convalescence was uneventful. On June 1, 1933, the serum calcium was 10.47 mg., serum phosphorus 3.85 mg. When last seen, on July 22, 1933, the patient had gained 12 pounds since operation and stated that she had not felt so well for years.

Gross Description: A pear-shaped, flattened, slightly firm, orange, encapsulated and pedunculated tumor 3 by 1.7 by 0.8 cm. The pedicle measures 1 by 1 by 0.4 cm. On one surface is a raised nodular area 3 mm. in diameter. The cut surface is uniformly yellowish brown and moist.

Microscopic Examination: The capsule is moderately thickened, measuring approximately 0.5 mm. It is composed of connective tissue strands between which are large numbers of congested vessels and many large fat cells.

This tumor is composed predominantly of two types of cells, similar to each other and both obviously related to the chief cell. There is, however, a definite dividing line between them.

The first type comprises a comparatively small proportion of the tumor and simulates the wasserhelle cell. The cell is polyhedral in shape with a fairly sharp pink outline and measures 10-14 microns in diameter. The nucleus is round, usually eccentric, sharply outlined, deeply basophilic, contains a moderate amount of chromatin and measures 7-9 microns in diameter. Except for a scanty, light pink, reticular cytoplasm, usually peripheral, most of the cell body is vacuolated. A few are completely vacuolated, but in general they have not reached the stage of true wasserhelle cells. No mitotic figures or multinucleated cells are seen. A small number of fat droplets are found in some of the cells. The stroma in this portion of the tumor is vascular. The capillaries are markedly congested with red cells and are so numerous that they often give the appearance of diffuse hemorrhage in between small groups or columns of cells. There are also larger endothelial-lined spaces, many of which are filled with a blue-staining, granular debris. No colloid or oxyphil cells are seen in this area.

The other and predominant part of the tumor is composed of slightly larger cells measuring up to 22 microns in diameter and averaging 15 (Fig. 20). Although these cells are not so sharply outlined as the others, many, nevertheless, have a sharp pink cell border. The nuclei, often multiple, are rounder, sharply outlined, for the most part centrally located, deeply basophilic and hyperchromatic and measure 8-10 microns in diameter. The most striking difference between the two cell types is in the cytoplasm, which shows almost no vacuolization and completely fills the cell body. There are no mitoses. The arrangement of the cells is also different. Here a large

proportion of the cells is arranged in well defined glands, averaging 65 microns in external diameter, with a lumen measuring about 22 microns in diameter. The single layer of lining cells is definitely cuboidal in character rather than polyhedral.

Many of the glands contain dark reddish pink, opaque, colloid-like masses, some completely filling the lumen, others only partially so. Some of the larger lumina show marginal vacuolization. Between these glands the stroma is scanty, but small endothelial-lined vessels, many filled with red cells and granular debris, are found. Occasional colloid droplets are seen in the connective tissue stroma. A small number of fat granules is seen in the cells, stroma and lumina. Although no true oxyphil cells are found, the predominant cell slightly suggests a transition stage to the oxyphil type.

CASE 19 (34-1526). *Clinical History:* T. G. Y., a male physician, 49 years of age, noted in January, 1932, the onset of malaise and muscle pains. In the course of 2 years he grew weaker and lost about 15 pounds in weight. Three months later he broke his clavicle during slight exertion. Slight nocturia had been noted for 5 years, but no gravel. Renal function test showed 30 per cent excretion. The serum calcium was 15.01 mg., phosphorus 2.61 mg., and phosphatase 14.1 units. X-ray showed changes in the bones characteristic of hyperparathyroidism and a questionable displacement of the esophagus to the left just above the sternal notch. On April 24, 1934, at operation a tumor arising close to the right inferior thyroid artery and extending backward and medially was subtotally resected, leaving a piece about twice the size of a normal parathyroid. The tumor had displaced the esophagus, as visualized in the X-ray film. The following day the serum calcium was 11.16 mg., phosphorus 1.25 mg., and phosphatase 13.2 units. When last seen, on June 15, 1934, he felt much better. The serum calcium was 8.04 mg., the phosphorus 3.82 mg.

Gross Description: A reddish brown, smooth surfaced, slightly lobulated and flattened, ovoid mass weighing 11.7 gm. and measuring 4 by 3 by 1.5 cm. Two small calcified areas 2-3 mm. in diameter project from the surface. One margin of the specimen is notched. The cut surface is homogeneously reddish brown and moist.

Microscopic Examination: A small rim of normal parathyroid tissue partially surrounds the tumor but is not separated from it by any definite fibrous tissue capsule.

The whole tumor is composed of a single type of cell which is polyhedral in shape, faintly outlined, and measures 11-16 microns in diameter. The cytoplasm is non-vacuolated, pinkish red, coarsely granular and completely fills all of the cell around the nucleus. The nucleus is round to ovoid, has a sharply demarcated basophilic out-

line, measures 6-8 microns in diameter and is usually eccentrically placed. The chromatin content is not very great and there are no mitoses. The cells contain no fat or glycogen. The appearance slightly resembles both the chief and pale oxyphil cell types, suggesting a transition stage.

The cells are closely packed in a manner similar to the arrangement of normal pale oxyphil cells but not, however, in islands or in palisade formation. The stroma contains no fat and is composed for the most part of large numbers of congested vessels, producing a pseudoglandular effect.

Summary of Cases 10 and 19 (Transition Oxyphil, Chief Cell Type)

These two tumors are composed predominantly of transition pale oxyphil cells, a stage between the chief and pale oxyphil cells (Fig. 20). They are arranged in glandular and pseudoglandular formation. No true oxyphil cells are present. One part of Case 10 has in addition a large area of transition wasserhelle cells.

CASE 4^{6,2} (32-3542). *Clinical History:* N. B., a female, 41 years of age, developed in 1925, following her fifth pregnancy, weakness in the back and knees and pain in the legs on walking. These symptoms increased with her sixth pregnancy in 1927, during which treatment for fallen arches was instituted. The diagnosis of "bone disease" made by her physician when she fractured the right femur in 1928 became obvious the following year when fractures of the right clavicle and later the right humerus occurred. In 1930 she entered the Massachusetts General Hospital where X-rays showed marked decalcification of the skeleton, cyst formation, old pathological fractures and a renal calculus. The serum calcium was 14.25 mg., phosphorus 2.3 mg. On Sept. 12, 1930, operation was done. The search, which was limited to the immediate region of the thyroid gland, failed to reveal a tumor but two normal parathyroid bodies were removed. A high calcium diet with viosterol gave improvement in symptoms and X-ray showed an increased deposit of calcium in the skull. As evidence of hyperparathyroidism persisted she returned to the hospital. At a second operation on Sept. 28, 1932, a large tumor was found behind the esophagus on the surface of the deep cervical fascia and a subtotal resection performed. She had moderately severe tetany during convalescence. In December, 1932, the serum calcium was 8 mg., phosphorus 4 mg., and phosphatase 4.3 units. When last seen, on Aug. 8, 1933, her anemia had improved and she felt much better.

Gross Description: An elongated, encapsulated, nodular mass of firm brown tissue measuring 3 by 1.5 by 0.9 cm. The cut surface is homogeneously reddish brown.

Microscopic Examination: The predominant cell in this case is the slightly enlarged chief cell (see Cases 6, 7 and 9). These cells are dis-

tributed in several ways. The major portion of them form the lining of large numbers of glands and cystic spaces (Fig. 21), which show great variations in size, some as small as 45 microns and others as large as 1 mm. Several of these spaces are partially or completely filled with a light pink, finely granular material, but many contain homogeneous, pink-staining material which slightly suggests colloid, an impression reinforced by the presence of marginal vacuolization, such as is seen in the hyperplastic thyroid. However, the light color and the lack of real density is more in favor of coagulation of the finely granular material rather than colloid. This same material, somewhat more deeply stained, is also found throughout the stroma, in many places completely obliterating the interstitial tissues. Some of these spaces are wholly or partially filled with red cells and occasional chief parathyroid cells, which may be desquamated lining cells. The lining is usually a single cell layer, but there is a fair number with two and even three layered linings. A few contain fat droplets.

Between these glandular structures are large collections of the same cells. Connective tissue stroma often containing small capillaries separates these collections of cells into small groups, many of which are pseudoglandular, and are composed on the average of about twenty-five to fifty cells, with occasional small lumina. Small groups of these cells, 5-10, arranged in gland formation resemble fetal adenomas of the thyroid. In several places the acinar cells show some degree of papillary infolding. In other areas the cells are arranged in undulating columns of three to four rows, the intervening stroma being composed of a fine reticulum. A tendency to palisading is barely recognizable. No mitoses are seen.

Scattered throughout all sections are pale oxyphil cells arranged in large groups varying in size from 0.1 mm. to 7-8 mm. The cells are slightly larger and less uniform than the normal oxyphil cell, varying from 8 to 17 microns. The cell outlines are fairly distinct, reddish pink and round to polyhedral in shape, clearer than the chief cell, but much less sharp than the wasserhelle cell. The nucleus is ovoid, deeply basophilic, sharply demarcated, centrally placed, hyperchromatic and fills about one-quarter of the cell volume. An occasional cell is multinucleated. Because of the large quantity of chromatin it is often difficult to distinguish a definite nucleolus. The cytoplasm is pink and granular, and usually fills the cell, although occasionally there is a small halo around the nucleus. Occasional, single dark oxy-

phil cells, usually located near the stroma, are found interspersed among the pale oxyphil groups.

The stroma of the glandular portion is composed of relatively dense fibrous tissue and large collections of colloid-like material. In the compact and pseudoglandular areas it is much less fibrous, but vascular.

CASE 13 (33-4182). *Clinical History:* A male, 22 years of age, was perfectly well until October, 1932, when he developed painless hematuria. In March, 1933, an attack of right renal colic was followed by the passage of a stone 2 weeks later, but several more attacks, one of them on the left side, pointed to the presence of additional stones. Physical examination was negative. The serum calcium was 15.78 mg., phosphorus 2.8 mg., phosphatase 4 units. Following X-ray of the urinary tract a right nephrolithotomy was performed. On Oct. 28, 1933, at operation a small parathyroid tumor under the upper pole of the left lobe of the thyroid was removed. On the fifth postoperative day the serum calcium was 10 mg., phosphorus 2.32 mg. When last seen, on Dec. 1, 1933, he was well, did not tire at the end of the day as he had done before, and felt much stronger.

Gross Description: A moderately soft, smooth surfaced, well encapsulated, slightly flattened, round tumor mass measuring approximately 1.7 cm. in diameter and weighing 2.1 gm. The surface is slightly mottled pale to orange-brown. The cut surface is moist, yellow to pinkish brown. The periphery is light brown to yellow.

Microscopic Examination: The capsule is quite thick, measuring up to 2 mm. in places, and is composed of fibrous connective tissue in which are numerous, endothelial-lined empty spaces. In addition there are many more unlined spaces that are partially or completely filled with parathyroid chief or wasserhelle cells. A tempting though uncertain interpretation is to regard these spaces as capsular lymphatics containing tumor cells. In any event there is definite evidence of parathyroid cells within the capsule. In one place there is a large group of wasserhelle cells just beneath the capsule. One end of this group of wasserhelle cells definitely invades the capsule and divides it for some distance into two layers. In the outermost layers of the capsule, and in one area very close to the outer surface of the capsule, there are chief cells arranged in glandular formation. Although this picture strongly suggests capsular invasion, it can also be interpreted as a rim of normal parathyroid tissue which has been markedly compressed by the tumor.

Just beneath the capsule are small and large foci of closely packed wasserhelle cells. The nuclei lie in the corner of the cell that is closest

to the stroma, giving the whole area a pattern similar to that seen in Case 16, where the whole gland has the same appearance. Many of the cells have no nuclei.

Except for the above mentioned capsule and subcapsular areas the specimen is composed of chief cells arranged in marked cystic and glandular formation, similar in parts to the glandular section of Case 4 (Figs. 22 and 23). There are no oxyphil cells. The cells are arranged in fairly compact masses and surround large numbers of cystic, irregular, papillary spaces, varying from 0.1 to 3 mm. in diameter. In many places these cystic spaces are lined by only a single layer of chief cells, but usually they are surrounded by the compact layers of the parenchymal chief cells. Some of these spaces are empty; many contain pink-staining granular debris; others are filled with red cells. The stroma is fairly abundant, contains many small vessels and no fat cells.

CASE 18 (34-1387). *Clinical History:* J. F., a female, 58 years of age. In 1924, 10 years before admission, the patient fractured her left femur after severe trauma, and had remained lame. In 1933 she fainted while at stool, fell, and broke the left femur again and also the left humerus. There were no genito-urinary symptoms. X-rays showed bone decalcification. The serum calcium was 11.36 mg., phosphorus 2.53 mg., and phosphatase 5.75 units. On April 13, 1934, at operation a tumor lying on the terminal divisions of the right inferior thyroid artery was resected. When last seen, on April 23, 1934, the calcium was 9.31 mg., phosphorus 3.32.

Gross Description: A flattened, almond-shaped, smooth surfaced mass 1.2 by 0.8 cm. by 0.4 cm. At one pole there is a semilunar area approximately 3 by 2 by 1 mm. which is yellowish brown and which is taken to be normal parathyroid tissue. The remainder of the specimen is dark purplish red and soft. This was thought to be tumor, although a hematoma in a normal gland could not be ruled out.

Microscopic Examination: A rim of normal parathyroid tissue surrounds the tumor. The dark purplish area observed grossly is a very vascular tumor. All the vascular channels, both small and large, are dilated and congested, producing a pseudo-acinar and in places papillary effect. The latter is further emphasized by the presence of numerous, irregularly shaped cystic spaces into which villus-like groups of cells project. Many of these spaces are empty, others filled with granular debris or red cells, and a few with colloid.

The cells are all of the chief and transition wasserhelle variety. No true wasserhelle cells are seen. Pale and dark oxyphil cells are absent. There are occasional intracellular fat droplets, but no intercellular fat globules such as are seen in the rim of normal parathyroid.

Summary of Cases 4, 13 and 18 (Glandular Cystic, Chief Cell Type)

These 3 cases are composed predominantly of slightly enlarged chief cells lining and surrounding numerous cystic and glandular spaces (Figs. 21, 22 and 23). This process is more prominent in Cases 4 and 13. Case 4 has in addition many islands of pale oxyphil cells. A rim of normal parathyroid tissue is present in Cases 13 and 18.

CASE 2 (32-157). *Clinical History:* M. L., a female, 60 years of age, experienced in 1928 a pain in the back which was intensified by motion, and followed by swelling and pain in the shoulder, knee and ankles. In February, 1931, a diagnosis of mild hypertrophic arthritis and osteomalacia was made. Serum calcium was 10.4 mg., phosphorus 3.6 mg. Treatment with a high calcium diet and viosterol resulted in considerable relief of symptoms, though the pain in the ankles and the aching in the knee continued and she was still subject to fatigue and inability to work. The diagnosis was temporarily changed to osteoporosis because of the consistently low phosphorus. Careful studies showed slight but constant elevation of serum calcium and low phosphorus, and a diagnosis of hyperparathyroidism was made. At operation, on Jan. 14, 1932, the right lower parathyroid body appeared considerably larger than normal and was removed. Convalescence was uneventful, without tetany. Serum calcium was 10.65 mg., phosphorus 3.68 mg. When last seen, on May 27, 1933, she was optimistic, felt much better and was working.

Gross Description: A smooth surfaced, moderately firm, ovoid, brownish mass measuring 10 by 5 by 4 mm.

Microscopic Examination: A section is taken through the whole mass. Under low power one sees a well circumscribed, encapsulated tumor, on one side of which is a peripheral zone of normal parathyroid tissue (Fig. 15). The tumor makes up about five-sixths of the specimen.

The capsule of the tumor is composed of a thin layer of acellular fibrous tissue. At one end of the section is a large, recent hemorrhagic area between the capsule of the tumor and the surrounding normal parathyroid tissue. In one place the hemorrhage has apparently broken through the capsule and is seen in the tumor.

The predominant cell is the typical wasserhelle cell (Fig. 16), measuring between 17 and 22 microns. The shape is usually poly-

hedral, but in the closely packed areas may be variable. The cell outline is a thin, sharp pink line, much more conspicuous than that of the normal parathyroid chief cells. The nucleus is eccentrically placed in one corner of the cell, is also sharply outlined and is deeply basophilic. It is round in shape, either clear or opaque, and measures about 8 microns. The nucleolus is just to one side of the center, is fairly conspicuous, and is surrounded by a large number of chromatin granules. Occasionally no nucleus is seen.

The cells are completely vacuolated, entirely lacking in demonstrable cytoplasm but contain moderate numbers of fat droplets. They can be regarded only as wasserhelle cells. In general the cells show no definite arrangement, although a single gland is noted. No mitotic figures are found.

In each section there are two to three large collections of cells which simulate the wasserhelle cells. They are about the same size and have a similar nucleus. The cell outline, however, is poorly defined and the cytoplasm is composed of a fine, reticular-like, pink cytoplasm in which are scattered, coarse, more brightly pink-stained granules. They have not the homogeneous cytoplasm of a true pale oxyphil cell, but may be a transition form (see Cases 10 and 19).

No oxyphil cells are found in the tumor, although they are present in fair numbers in the surrounding normal parathyroid tissue.

The stroma is scant and made up almost solely of fine capillaries and occasional small, vacuolated spaces, 11-15 microns in diameter, which contain homogeneous, pink-staining, colloid-like masses. One portion of the tumor contains a number of large fat cells similar to those seen in the surrounding normal parathyroid tissue.

Summary of Case 2 (Neoplasia: Wasserhelle, Generalized)

An encapsulated tumor composed predominantly of wasserhelle cells, scattered among which are a few large collections of probable transitional oxyphil cells. There are no mitoses or multinucleated cells. There is a rim of normal parathyroid around a portion of the tumor (Figs. 15 and 16).

CASE 5 (32-3594). *Clinical History:* R. T., a female, 55 years of age. In 1922 the patient developed attacks of severe pain in the right flank, radiating to the epigastrium, for which the gall-bladder was removed in 1927. The attacks not only were not relieved but became more severe. She was easily fatigued. In September, 1932, at the Massachusetts General Hospital a stone, shown by

X-ray in the pelvis of the right kidney, was removed by pyelotomy. Routine blood chemistry studies showed the serum calcium to be 13.2 mg., phosphorus 2.78 mg., phosphatase 5 units. On Oct. 3, 1932, a parathyroid tumor below the left lower pole of the thyroid was resected. A mild tetany was present postoperatively. When last seen, on Dec. 9, 1932, the serum calcium was 10.34 mg., phosphorus 3.71.

Gross Description: A slightly firm, reddish, and in places orange, slightly ecchymotic, encapsulated tumor measuring 1.5 by 1 by 1 cm. The cut surface is homogeneous, smooth, glistening and orange to reddish gray.

Microscopic Examination: The capsule is thin. Under low power one can see fairly large circumscribed masses of wasserhelle cells scattered at intervals through the tumor, which is elsewhere composed of chief cells (Fig. 24). These masses vary in size from 0.1 to 1.5 mm. in diameter. The subcapsular portion contains large collections of dark oxyphil cells.

The wasserhelle cells probably make up more than half the tumor. They are polyhedral, closely packed and measure 11–20 microns in diameter (Fig. 24). Their cell outlines are thicker than normal, reddish pink and ragged, but are easily seen because of the vacuolated cytoplasm. The nucleus is large, measuring 8–11 microns, eccentrically placed, round, sharply circumscribed, deeply basophilic, and so packed with chromatin that in many instances the nucleolus cannot be made out. The cytoplasm for the most part is completely vacuolated. A few cells contain pink-staining, coarsely granular debris, others lighter but brighter pink, homogeneous clear droplets 3–5 microns in diameter. No fat droplets are seen. There are no mitoses. Scattered among these cells are a few that have a light pink granular cytoplasm. These may well be transitions between the chief cell and the fully developed wasserhelle cell. No true oxyphil cells are found in the wasserhelle groups. The stroma between these wasserhelle cells is scant, but where it is present definite endothelial-lined vessels containing red blood cells are found. A few of the colloid-like droplets are also found in the stroma. Scattered throughout are irregularly shaped small spaces, which vary from 15 to 90 microns, most of them empty, but some containing pink-staining debris and others red blood cells.

Around the wasserhelle groups are slightly enlarged chief cells arranged for the most part in compact masses, in a few areas toward

the periphery in well formed glands with walls one to three cells deep, many of which are filled with red cells. Near the periphery, where the chief cells predominate, the stroma is markedly congested and contains large globules of fat.

Groups of typical, normal dark oxyphil cells are found close to the periphery, while single ones are distributed throughout the gland, except among the wasserhelle cells.

Summary of Case 5 (Neoplasia: Wasserhelle, Focal)

An encapsulated tumor composed of both chief and wasserhelle cells (Fig. 24). The latter are arranged in circumscribed masses making up more than half the tumor; the former are smaller and arranged in compact masses and in a few places in glands. There is very little fat in either type of cell.

CASE 20 (34-2321). *Clinical History:* N. M. K., a female, 36 years of age, in 1928 had her first attack of renal colic followed by a similar attack 3 months later. In 1930 stones were removed from the left kidney and right ureter but she continued to have attacks of renal colic. In 1933 she was delivered of a healthy full term child, following which she passed many small stones and developed polydipsia. There was no history of bone or joint pains or loss of weight. She entered the Massachusetts General Hospital in May, 1934. The urine was loaded with white and red blood cells, and had a fixed low specific gravity. The phenolsulphonephthalein test showed 40 per cent excretion. The serum calcium was 12.16 mg., phosphorus 3.27 mg. and the phosphatase 6-8 units. On June 13, 1934, at operation both upper parathyroid glands were found enlarged and were removed; both lowers were normal in size and a biopsy of each was taken. Serum calcium and phosphorus taken 7 hours postoperatively were 9.56 mg. and 2 mg. respectively. Mild tetany developed on the second postoperative day. When discharged, on June 25, 1934, the serum calcium was 10.62 mg., the serum phosphorus 4.52 mg.

Gross Description: (Right Upper): A yellowish brown, with reddish mottling, encapsulated mass 1.3 by 0.7 by 0.3 cm., weighing 0.28 gm.

(Left Upper): A light brown, flattened, round encapsulated mass measuring 1 by 0.8 by 0.3 cm. and weighing 0.3 gm. The biopsies from the lower glands are light brown in color and measure about 1 mm. in diameter.

Microscopic Examination: (Right Upper): The thin capsule is composed of acellular fibrous connective tissue and no rim of normal parathyroid tissue can be demonstrated around the tumor. The blood vessels are markedly congested and in addition there are many

large extravascular collections of red blood cells. The predominant cell throughout the tumor is a typical enlarged chief cell with the usual ill-defined cell outline and large hyperchromatic round nucleus, similar to that seen in Case 6. The cells average 10 microns in diameter, the nuclei 7 microns. The arrangement is protean. There are compact masses, anastomosing cords running between dilated capillaries of sinusoidal appearance, and large areas of gland formation. Many of the glands are lined with chief cells resembling the bulk of the tumor. Other glands, however, are of a totally different appearance, resembling none that we have seen in any of our other cases.* The lining cells here do not resemble chief cells. They are columnar in shape with basal nuclei and a rather localized area of vacuolization which does not surround the nucleus but always lies in the opposite pole of the cell toward the lumen of the gland. The appearance closely simulates the duct of a mucous gland lined by goblet cells. Both types of glands are filled with red cells (Fig. 26). Wasserhelle cells are completely, and pale and dark oxyphil cells practically, absent. In an occasional area normal pale oxyphil cells surround some of the glands.

The stroma is for the most part scant, with wide and thin-walled capillaries and many foci of hemorrhage, but in a few places several isolated cells are surrounded by irregular areas of almost acellular, richly collagenous fibrous tissue. There are no intercellular fat droplets. A small amount of colloid is present.

(*Left Upper*): This gland is quite different from the right upper (Figs. 25 and 26). In this instance a rim of normal parathyroid tissue containing many large fat cells in its stroma surrounds the tumor and is separated from it by a fairly thick connective tissue capsule. The cells are all of the same type — the transitional wasserhelle cell. There are a few intracellular fat droplets. Glycogen is present in normal amounts. Except for the absence of true wasserhelle cells, this specimen is similar to Case 12. There are no pale oxyphil cells and only an occasional normal dark oxyphil cell. All the cells are massed compactly together with little stroma. In a few places near the periphery there is a slight tendency to pseudo-alveolar arrangement and a slight resemblance to the pattern observed in the hyperplastic group.

* Case 24 in the Massachusetts General Hospital series, which is not reported in this paper because operation was performed after the paper was submitted, shows a single adenoma with this same histological picture.

The biopsies from the two lower glands show normal parathyroid tissue.

CASE 21 (34-2362). *Clinical History*: E. T., a female, 35 years of age, had an attack of left renal colic followed by the passage of a stone in November, 1933. Three months later several stones were removed at a local hospital. X-rays of the skeleton were normal. Following operation she felt well except for easy fatiguability and occasional low backache. She entered the Massachusetts General Hospital in June, 1934. Physical examination was negative. A renal function test showed 65 per cent excretion in 2 hours. The serum calcium was 11.92 mg., serum phosphorus 2.86 mg. On June 16, 1934, at operation the right lower parathyroid was found enlarged and was resected. The right upper was about the same size and a small biopsy of it was taken. No parathyroid tissue was found on the left side, even after the left lobe of the thyroid had been removed and carefully examined. On June 17th the serum calcium was 8.6 mg., phosphorus 3 mg. On the second postoperative day she developed mild tetany which lasted only a few days. When discharged, on June 26, 1934, the serum calcium was 10.34 mg., phosphorus 2.9 mg. A stone was still present in one kidney.

Gross Description: (*Right Lower*): A moderately firm, yellowish brown encapsulated mass 1 by 0.6 by 0.3 cm. The cut surface is uniformly yellowish brown. (*Right Upper*): A small biopsy approximately 2 mm. in diameter.

Microscopic Examination: (*Right Lower*): Around one edge of the tumor is a small rim of normal parathyroid tissue composed of chief cells and several large fat globules. The capsule of the tumor is thin. The tumor is composed for the most part of two types of cells, chief and pale oxyphil, with a slight predominance of the oxyphils. The chief cells of the tumor measure about 10-12 microns in diameter, the nuclei 8-10 microns; those in the rim of normal tissue measure 7-8 microns with nuclei of 5-6 microns. The cytoplasm is only slightly vacuolated and contains an occasional tiny fat granule and a normal amount of glycogen. There are no extracellular fat globules. The arrangement of the tumor cells is pseudoglandular. In many places, however, pale oxyphil cells are adjacent to chief cells, but almost all the latter are true chief cells and not in transitional stages to pale oxyphils. Glycogen is present in normal amounts. In addition to the scattered, single, pale oxyphil cells many of them are arranged in large islands. This finding is unusual in a person 35 years of age.

The biopsy of the upper gland shows a similar picture.

Summary of Cases 20 and 21 (Neoplasia: Multiple)

In each of the 2 cases two tumors were found composed predominantly of chief cells. In Case 20 one is definitely glandular; the

other is non-glandular, made up wholly of the transition wasserhelle cell and has a rim of normal parathyroid tissue. In Case 21 both tumors have the same appearance and contain many pale oxyphil cells, both single and in large groups.

REVIEW OF THE LITERATURE

One hundred and sixty cases of probable hyperparathyroidism have been collected from the literature and the significant data tabulated (Table VI, see page 50). We have attempted to include all tumors and tumor-like enlargements of the parathyroid glands but have excluded from the series cases of osteomalacia, rickets, and primary nephritis in which slight secondary parathyroid hyperplasia is frequent. Rigid proof of hyperparathyroidism is often lacking, but in cases of marked parathyroid enlargement the burden of proof rests on the disclaimer.

Many of the case reports are regrettably incomplete, either in the clinical or in the anatomical details, and this considerably limits the value of the table. Knowledge of the syndrome of hyperparathyroidism has developed slowly and although the association of a parathyroid tumor with bone lesions was reported as early as 1903 by Askanaazy,¹⁰ it was not until 1913 that the combination began to be noted by a significant proportion of writers on the subject. Even at the present time the association with renal stones is still largely unrecognized and the experience in our clinic strongly indicates that more attention devoted to this phase of the disease will greatly increase the proportion of cases in which renal calculi are reported.

Statistical Data

Age

Including our series the ages are stated in 176 cases.

TABLE I

Distribution per Decade

Age in years	0-9	10-19	20-29	30-39	40-49	50-59	60-69	70-79	80-89
Total cases	0	11	28	29	44	43	12	8	1
Adenomas	0	10	25	27	37	38	11	5	1
Hyperplasias . . .	0	1	3	2	7	5	1	3	0

The highest incidence of hyperparathyroidism is between 40 and 60 years of age. There is no significant difference in this regard between the group of localized tumors and the group of diffuse hyperplasias. Although the youngest case recorded is 13 years of age (our Case 3), the symptoms in this patient were clearly of at least 4 years duration, so that the occurrence of the disease in the first decade is to be expected in rare instances.

Sex

Sex is reported in 174 cases.

TABLE II

<i>Sex Incidence</i>		
	Females	Males
Total	122	52
Adenomas	108	45
Hyperplasias	14	7

The predominance of females over males is evident in both groups, being in a ratio of approximately two and a half to one for the adenomas and exactly two to one for the hyperplasias.

Number of Glands Enlarged

TABLE III

<i>Data on Enlarged Glands</i>	
Available cases	185
Single gland enlarged	146
Multiple enlargements	39
(a) Two glands	25
(b) Three glands	1
(c) Four glands	13

Site

The data are distinctly inadequate. Of 119 single tumors in which location is recorded, 56 were on the right and 47 on the left. In 76 cases an attempt was made at more accurate localization and of these, 12 adenomas were found in one of the upper glands and 64 in one of the lower glands. This five to one ratio is of evident surgical value. Tumors of aberrant parathyroid glands are by no means infrequent — 6 have been found within the thyroid, 2 in the thymus,

and 9 have been described as retrosternal. Where two glands have been reported as enlarged any combination is possible, but again the lowers have been more frequently involved than the uppers.

Size

The sizes of the tumors vary over an extreme range. Symptoms of hyperparathyroidism have been recorded with a tumor only twice the size of a normal gland. At the other end of the scale tumors have been recorded weighing as much as 300 gm. and Benjamins¹⁸ described one as large as a "child's head."

Incidence of Osteitis Fibrosa and Renal Stones

The presence of bone lesions is recorded in 116 of the 160 cases collected in Table VI, but in only 4 of the remainder were they specifically stated to be absent. A glance at the table where the cases have been listed in the order of their publication will show how rarely the relation was recognized by the earlier writers. Since an analogous situation still prevails in regard to renal stone formation, we have listed our cases separately in the following table. The value of routine blood calcium and phosphorus determinations in all cases of renal stone formation (Albright *et al.*⁵) is at once evident when the percentage of calculi in this series is compared with that in the previously reported cases. We have included only the cases in which a definite statement in regard to bone lesions was recorded.

TABLE IV

Incidence of Osteitis Fibrosa and Renal Stones

	Present series		Cases in literature	
	No.	% of total (25)	No.	% of total (119)
Osteitis fibrosa alone	5	20	70	58.8
Renal stones alone	11	44	3	2.52
Osteitis fibrosa plus stone	9	36	46	38.6

When our own series of cases is divided into the hyperplastic and the adenomatous groups, 13 of the latter showed bone lesions, whereas the 5 clear cell hyperplasias fall into the group of renal stones without bone changes and only Case 23A, the chief cell hyperplasia, showed significant bone lesions. That this is the result of chance

sampling in too small a series of cases is at once apparent by reference to the group of 14 clear cell hyperplasias collected from the literature, all but 1 of which showed bone lesions. It is evident that either type of hyperparathyroidism may be associated with stone formation only, bone changes only, or the combination. As a rule stone formation comes first and bone lesions follow only after a period of years. When the average duration of symptoms in our cases showing only renal stones is calculated, it is 3.2 years, whereas the average duration in the cases with classical bone lesions is 8.6 years.

CLASSIFICATION

As we have already briefly outlined in the introduction to our own series of case reports, we believe the fundamental line of division in the pathology of hyperparathyroidism lies between diffuse hyperplasia of all the parathyroid tissue and localized proliferation of only a portion, the remaining glandular tissue being histologically normal. In the first type diffuse enlargement of all the glands is to be expected; in the second type one or at most two will be involved. The division, however, judging from our own experience and the reports of others, cannot safely be made upon the number of grossly enlarged glands in each case. The degree of enlargement of individual glands varies greatly and though slight enlargement of every gland is probably always present in hyperplasia, the swelling of one or two glands may be so predominant that minor enlargement of the others might readily be overlooked, particularly under the exigencies of a surgical operation. In cases, however, where portions of all the parathyroids have been examined microscopically, as in Cases 17, 23, 25 and 23A of our series, and 32, 36 and 53 from the literature, the uniformity in histological appearance of all the glands, whether large or small, is at once apparent.

Fortunately, however, the histological picture of the hyperplastic gland, at least of the more common wasserhelle type, is so characteristic, so different from anything we have seen in the cases of single tumor formation that we believe a diagnosis of hyperplasia should be possible as a rule from the histological examination of a single gland, even from a frozen section during an operation. The uniform, giant sized clear cells, the acinar arrangement, the basal orientation of the nuclei form a readily recognizable picture (Figs. 8-11).

That hyperplasia of a different type, uniform proliferation of chief cells without significant vacuolization, can occur is shown by our Case 23A (Figs. 12 and 13) and by Cases 29 and 61 from the literature. Hyperplasia of this type, marked enough to cause significant tumor-like enlargement of the glands, is evidently rare since including our own case we have been able to find only three examples.

Another potential source of error in classification, if gross enlargement only is considered, lies in the confusion of multiple neoplasms with hyperplasias. Numbers 20 and 21 of our series are, we believe, cases in point. In Case 20 all four parathyroids were exposed at operation, two which were enlarged were resected and from the other two, which appeared normal, biopsies were taken. The biopsies show normal parathyroid tissue. The two enlarged glands are shown in Figs. 25 and 26. In contrast to the hyperplastic cases where every gland presents a uniform appearance, one of these tumors is frankly glandular in character, the other consists of solid masses of chief cells without evident arrangement. In Case 21 two tumors of identical appearance, the familiar chief cell adenoma, were found, but a rim of normal gland about one of the tumors definitely rules out diffuse hyperplasia. Bergstrand²¹ twice demonstrated a rim of normal gland about each of a pair of localized tumors.

An attempt to classify the cases from the literature is admittedly dangerous but by limiting ourselves to cases in which a reasonably complete histological description is recorded or in which adequate illustrations allow us to judge for ourselves, we believe that a fairly accurate classification is possible. That several of the cases may have been misplaced is frankly admitted. One hundred and twenty-eight cases from the literature have been utilized. To these have been added, besides our own series of 25, an additional 9 unreported cases from other hospitals which the authors have been given the privilege of examining histologically.* In compiling the table single glandular enlargement has been automatically placed in the neoplastic group, cases with three or four enlarged glands in the hyperplastic one. Where two glands were enlarged we have attempted classification on the basis of the histological features. Five cases of multiple enlargement, 37, 62, 94, 141 and 159, we have felt unable to classify.

* These 9 cases, all single tumors, are distributed as follows: chief cell alone 3, chief cell with giant forms 1, transition wasserhelle 2, glandular and cystic 1, wasserhelle generalized 1.

The localized enlargements or neoplasms have been subdivided first into single and multiple groups and then classified on purely morphological grounds. The sequence of the classification (Table V)

TABLE V
Classification of Cases

	Case Nos. in our series	No. in our series	No. in litera- ture	Percent of total
A. Hyperplasia (multiple) (22 cases)				13.6
1. Wasserhelle, generalized	15, 16, 17, 23, 25	5	14*	
2. Chief	23A	1	2†	
B. Neoplasia (140 cases)				86.4
1. Single (128 cases)				
(a) Chief cell types (114 cases)				
(1) Chief cell alone	6, 7, 9	3	59	
(2) Chief cell with giant forms	1, 11	2	3	
(3) Transition wasserhelle	3, 22, 8, 12, 14	5	17	
(4) Transition oxyphil	10, 19	2	6	
(5) Glandular and cystic	4, 13, 18	3	14	
(b) Wasserhelle cell types (14 cases)				
(1) Generalized	2	1	11	
(2) Focal	5	1	1	
2. Multiple (12 cases)				
(a) Chief cell types	20, 21	2	7	
(b) ? Oxyphil cell			3	
		25	137	

* Case Nos. 22, 23, 25, 32, 36, 53, 89, 92, 98, 123, 125, 126, 127, 155.

† Case Nos. 29, 61.

follows the order in which the case reports have been presented above.

Out of a total of 161 cases 22 or 13.6 per cent appear to belong in

the hyperplastic group, 19 of which are in the wasserhelle type as against 3 in the chief cell type. The far commoner localized tumor formation is represented by 140 cases, 86.4 per cent of the total, 130 of them single tumors, 10 of them multiple. In the single tumors the chief cell with its transition forms accounts for at least 90 per cent.

DISCUSSION

Hyperplasia versus Neoplasia

Since the recognition of the syndrome of hyperparathyroidism, the question whether to regard the proliferative changes in the glands as hyperplastic or neoplastic has been a matter of controversy which the paucity of available evidence served only to stimulate. The demonstration of a distinct group of cases characterized by diffuse uniform involvement of all parathyroid tissue is, we believe, the first unequivocal evidence bearing on this issue. Such diffuse involvement points so strongly to hyperplasia dependent on a generalized humoral stimulus (possibly though improbably with mediation of the nervous system), and so strongly against a local autonomy that neoplasia cannot be seriously considered. The recognition, moreover, of at least two distinct histological types of hyperplasia not only suggests the interesting possibility of multiple potential stimulating factors but confirms the essential pattern of the hyperplastic lesion — the uniform diffuse involvement of all the glandular tissue. The analogy to exophthalmic goiter is of course apparent.

In sharp contrast to this relatively uncommon type of case stands the far more usual type of a localized proliferative process which leaves entirely uninvolved the remaining glandular tissue. Let us first consider several suggestive but inconclusive histological criteria favoring the neoplastic origin of the localized tumors (which our cases illustrate). All of them have been repeatedly cited before, but they rise in importance by their comparison with known hyperplastic lesions.

Cell size and number of nuclei fail to provide distinguishing criteria since rather surprisingly the hyperplastic group provides the largest cells and the most frequently multinucleated. But among the group of localized tumors an occasional example is encountered of gigantism of the nuclei up to 20 microns (Fig. 18) associated with an irregular multilobulated outline and extreme hyperchromatism,

which has no parallel in the hyperplastic state and which has a certain *prima facie* neoplastic quality.

In comparison with the rather monotonous uniformity as a group and also from field to field of the hyperplastic cases the localized tumors present a protean picture not merely as a group, but also at times within the dimensions of a low power field of a single tumor. Tumors may be made up almost solely of chief cells (Fig. 17), of fully developed wasserhelle cells (Fig. 16), of transitional wasserhelle (Fig. 19), of transitional oxyphil cells (Fig. 20), or any combination of these elements. Well developed gland formation will be present in one area, broad anastomosing cords in another and solid patternless cell masses in a third.

Fibrous stroma which is scant but uniform in its distribution in the normal gland increases in the hyperplastic gland to strands of uniform width which surround each acinus and sharply demarcate it from its neighbors. In the localized tumors, in contrast, it is markedly irregular in character and distribution, here abundant and richly collagenous, there tenuous and barely demonstrable. Blood vessels which are constant in size in the normal gland, rather uniformly increased in number and diameter in the hyperplastic gland, become irregular in caliber and distribution in the tumor nodules. A sinusoidal dilatation of capillary vessels is a frequent abnormality. Endothelial-lined spaces, probably lymphatics, which are undemonstrable in normal and hyperplastic glands, are frequently conspicuously dilated.

The crux of the argument rests, in our opinion, in the localized character of the proliferative process. Our own experience indicates this is frequently limited not merely to one gland but to a portion of a single gland. In 8 of our 19 adenomatous cases we have been able to demonstrate a rim of normal parathyroid tissue on one margin of the tumor. That this has been noted in the literature on only eight occasions can be explained we feel on two bases: (1) it has not been systematically looked for, and (2) our cases for the most part are early ones with relatively smaller tumors than the majority that have been reported. It is obvious that the mathematical chances of demonstrating a small fragment of normal parathyroid tissue in or on the capsule of a tumor diminish rapidly with increase in size of the tumor. Partial or total atrophy of the normal remnants is, moreover, not improbable with tumors of large size.

If an external humoral stimulus to overgrowth is present in these cases, it must be of minimal importance compared with the local autonomous factor which determines the site of the proliferative activity. Moreover, if the newgrowth were in response to a persisting outside stimulus, surgical removal should logically be followed by reasonably prompt recurrence of the growth process in one of the other remaining glands. Surgical experience does not support this in a wide experience with the localized tumors. In the realm of typical hyperplasias experience is still limited, but the short follow-up on our Cases 15 and 23 strongly suggests an extrinsic factor. In Case 23, three enlarged glands and a biopsy of a normal sized fourth gland were removed, following which the serum calcium fell from 13.1 to 10.18 mg. Three months later the serum calcium was 11.96 mg. Case 15, in which three enlarged glands were removed, with a drop of serum calcium to 11.4 mg., is awaiting further treatment with a serum calcium that has risen once more to 13.8 mg.*

We can, therefore, distinguish on the available evidence between two groups of proliferative changes in the parathyroid glands, one primarily dependent on an external, continuous stimulus, the second independent, as far as can be made out, of such a stimulus, determined in its localization and duration by local autonomous factors which can be extirpated by local surgical removal. This second type of proliferation, an essentially autonomous newgrowth, falls within the accepted limits of the term neoplasia.

*Comparison of the Size of Hyperplastic Glands and Adenomas
with the Degree of Hyperparathyroidism*

The degree of hypertrophy of glandular tissue in cases of parathyroid hyperplasia is in itself worthy of attention. The material removed from Case 16 of our series, the severest of our hyperplastic cases, amounted to 15.6 gm. This is approximately one hundred times the weight of the total normal parathyroid tissue. A degree of hyperplasia equal to this is totally unparalleled in human pathology. In exophthalmic goiter, in mazoplasia or in prostatic hyperplasia five or tenfold hypertrophy would be unusual. Even lactation hypertrophy of the breast is left far behind.

* A fourth gland in this case was not found and all of the third gland, except for a piece twice the size of a normal gland, was resected.

In the case of parathyroid adenomas still greater variations occur. In our Case 1 the tumor weighed 53 gm., approximately four hundred times the normal, and much larger tumors are on record. If this new-formed glandular tissue functioned in proportion to its size, some individuals would die of parathyroid poisoning, like that so easily produced in animals with parathormone,⁷⁷ unless some compensating mechanism were brought into play. An answer to the problem must await biological assays of material from both hyperplastic and adenomatous glands.

That a roughly quantitative relation between size of tumor and degree of hyperfunction exists is apparent from the following figures. Since weights were lacking on several of the cases, we have compared the volumes.

(5 cases) Blood calcium less than 12 mg. Average volume 255 cmm.

(9 cases) Blood calcium 12 to 14 mg. Average volume 3830 cmm.

(8 cases) Blood calcium greater than 14 mg. Average volume 16,000 cmm.

It is evident that as the size of the tumor increases the proportional effect of unit weight on the blood calcium becomes rapidly less and less. In fact in the hyperplastic cases the relation appeared to approach a logarithmic function. The hyperplasias, as might be expected from their histological uniformity, show a more nearly mathematical relation. The adenomas in contrast show far wider variations. All attempts to correlate the degree of hyperfunction with the histology of the tumors have proved fruitless.

Function

Throughout the history of endocrinology the study of tumors of the ductless glands has played an important rôle, sometimes pointing the path to chemical researches, as in the case of the pituitary adenomas, sometimes bringing up the rear to give final confirmation to an already well understood mechanism, as in the pancreatic islet adenomas. The multiplicity of cell types in the parathyroid glands naturally makes one think of the pituitary. Does the study of parathyroid tumors aid us in understanding the histophysiology of the normal organ?

In the normal gland there is general agreement, and our own studies are in accord, that glycogen can invariably be found at any age and is present in every type of cell except the fully developed

oxyphil. Unfortunately, material was not suitably preserved for glycogen stains in all of our cases, but we have available at least 1 case of each type and as yet have not failed to demonstrate at least some granules. As a general rule it is less abundant in both the tumors and the hyperplasias than in the normal gland (in adenomas with normal tissue in the capsule this is often strikingly apparent) but in at least some cells of every tumor it will be found. It is apt to be most evident in the cells that approach most nearly the normal chief cell in appearance. It is present in the wasserhelle cells and it is least marked or absent in the cells that most nearly approach the normal oxyphils. We can, therefore, say that glycogen has been found present whenever sought for in every case of hyperfunction. This reinforces the fact that it is invariably present in the normal gland and suggests that glycogen is in some way necessary to the elaboration of the specific hormone.

Fat droplets within the parenchymal cells are in contrast entirely lacking in the normal glands of children and cannot, therefore, be necessary to the elaboration of the hormone by which the calcium balance is maintained. In confirmation, intracellular fat has been present in some of our adenomas, absent in others.

Fat cells in the stroma cannot seriously be considered to have any direct bearing on the function of the gland. They remind one naturally of the bone marrow, and their relative independence of the state of nourishment of the individual might suggest a similar function, a readily resorbable tissue permitting rapid and facile hyperplasia. In hyperplastic glands it entirely disappears; in the adenomas it is usually absent though occasional fat cells can be found.

Have the oxyphil cells a function? Certainly their presence is not necessary to the normal functioning of the gland since they appear only with middle age and do not become numerous until advanced life. Oxyphil cells are wholly absent from the hyperplastic physiologically overactive gland. True oxyphils may be wholly absent from the adenomas; they may be scattered in small numbers much as they are distributed in the normal gland of early adult life; they may be present in localized collections similar to the oxyphil islands of old age. Transition oxyphils, according to our classification cells with large amounts of homogeneous red cytoplasm but still with traces of vacuolization about the nucleus, may make up the bulk of a tumor, but among them cells which closely approach the chief cell type have

always been found. We have been unable to classify any of our cases as a true oxyphil adenoma and although cases have been so classified by other authors, we have not found their descriptions convincing. Turnbull's case of oxyphil adenoma (Case 93), for instance, clearly shows from an illustration some degree of halo formation about the nucleus. He himself speaks of the presence of glycogen and fat, though he has never been able to demonstrate these substances in the typical oxyphil cells of normal glands. We feel, therefore, that it can fairly be said that histological evidence fails to support the concept of the elaboration of parathyroid hormone by the oxyphil cells. The frequency of these cells in tumors, even in young people, their absence in hyperplasias, their increase under normal conditions in old age, all point toward an involution phenomenon. The possibility of another function, unconnected with the calcium metabolism cannot be ruled out, but we have found no evidence for it.

Interrelation Between Cells

Since Welsh's fundamental study of the histology of the parathyroid glands, the interrelation of the various cell types has been under discussion. He sharply separated the oxyphil cell but believed in transitions between the water-clear and the chief cell, though he considered the former the more primitive type, in contrast to later workers, such as Getzowa,⁵⁶ who have felt that if one were derived from the other, it was the chief cell which was the primitive form.

With Kurokawa⁸⁵ the possibility that the oxyphil also was derived from the chief cell was considered and various transition forms were described. Hunter and Turnbull⁸⁰ have developed this concept, stating that "the oxyphil cells are principal cells in which the cytoplasm has been so charged with oxyphil granules that the basophil net has been more or less completely reduced to a limiting membrane."

Does a study of the tumors of the parathyroid glands contribute any evidence for or against a monophyletic development of the various cell types? As Hunter and Turnbull have pointed out, the normal evolution of the gland in fetal and adolescent life, starting only with chief cells, with successive development of water-clear cells, of pale oxyphils and dark oxyphils with the simultaneous appearance of transition forms argues for a single fundamental cell type. The clear

TABLE VI

Summary of Case Reports from the Literature

Author	Year	Age in yrs.	Sex	No. of tu- mors	Site	Size in cm.	Weight in gm.	Bone change	Renal stone	Cell type	Comment
1. de Santi	1900	58	M	1		Very large				No histology	
2. Benjamins	1902	57	M	1	R	Child's head				Chief	
3. Askanazy	1903	51	F	1	L	4.5 × 2		+	+	Chief	
4. Erdheim	1903	18		1	RL	2.5 × 1.5 × 1.5				Chief	
5. MacCallum	1905	26	M	1	RL	2 × 2 × 2				Chief (occasional wasserhelle)	
6. Hulst	1905	old	F	1	In thy- roid	2.5 × 2.5 × 2				Transition wasserhelle	
7. Weichselbaum ...	1906		M	1	RL	2.5 × 1.5 × 1.5					Died of pneumonia
8. Weichselbaum ...	1906		F	1	LU	4.3 × 3.6 × 1				? Glandular and cystic	Died of pneumonia
9. Langhans	1907	58	M	1		13 × 9 × 8				Wasserhelle generalized	Operation for goiter
10. Langhans	1907	60	F	1		10 × 8 × 2				Wasserhelle generalized	Operation for goiter
11. von Verebely	1907	42	F	1	RL	2.5 × 1.8 × 1.5				Chief (occasional wasserhelle)	
12. von Verebely	1907	56	M	1	LL	1.2 × 0.8 × 0.3				Wasserhelle generalized	
13. Schmorl	1907	48	F	1	LU	2.8 × 1.8 × 0.5		+		Chief	
14. Thompson and Harris	1908	23	F	1		15 × 10 × 6	250			Chief	Operation for goiter

15. Bérard and Alamartine ...	1908	43	F	1	L	2 × 1.5				Chief (occasional wasser- helle)	
16. Pepere	1907	40	F	1	LU	Apple sized				Chief	
17. Da Costa	1909	32	F	1	R	Orange sized				Glandular and cystic	Operation for goiter
18. Strada	1909	54	F	1	R	2.8 × 1.4 × 0.9	+	+		Chief (occasional wasser- helle)	4 other glands slightly enlarged. ? osteomalacia
19. Claude and Schmiegeld ...	1909	85	M	1	RL	1.5 × 0.7 × 0.5				Wasserhelle generalized	Epilepsy
20. da Costa	1909	50	F	1	RL					Glandular and cystic	
21. Gussio	1910	30	F	1	L	Small mandarin				Chief	
22. Möller	1911	72	F	2	LU RU	2 × 1.2 × 1 0.8 × 0.6 × 0.4				Wasserhelle generalized	Lowens not found
23. Möller	1911	46	F	2	LU RU	4.5 × 0.5 × 0.5 4.5 × 0.5 × 0.5				Wasserhelle generalized	Miliary tuberculosis
24. Ikonnikoff	1912	57	F	1	In thy- roid	Mandarin				Chief	
25. Gjestland	1912	75	M	2	RL LL	4 × 1 Hazel nut	o	o		Wasserhelle generalized	Uppers slightly enlarged
26. Schmorl	1913	72	M	1	U		?	Pag- et's		No histology	
27. Molineus	1913	74	F	1	R	2.7 × 1.7 × 0.7	+			Transition wasserhelle	

R = right; L = left or lower; U = upper.

TABLE VI (continued)

Author	Year	Age in yrs.	Sex	No. of tumors	Site	Size in cm.	Weight in gm.	Bone change	Renal stone	Cell type	Comment
28. Molineux	1913	59	F	2	LU RL	2.7 × 1.8 × 0.8 2.7 × 1.8 × 0.8		+		Chief (? transition wasserhelle)	2 others normal
29. Molineux	1913	48	F	4	RU RL LU LL	1.8 × 1.2 × 0.2 1.9 × 1.7 × 0.8 1.6 × 0.4 × 0.3 2.3 × 1.1 × 1.2		+		Chief (papillary)	3 others normal Called osteomalacia
30. Paltauf	1913	51	F	1	RL	3.5 × 3.5 × 2		+	+	Chief	Paralysis agitans
31. Harbitz	1915	26	F	1	L	4 × 1 Pea 2 × 2.5 1 × 1.2				Wasserhelle generalized	Died of tuberculosis
32. Harbitz	1915	75	M	4	RU RL LU LL	11 × 5				? transition oxyphil	3 others normal
33. Harbitz	1915	32	F	1	LL	7 × 4 × 1.5			o	Glandular and cystic	3 others normal
34. Maresch	1916	69	M	1	L	4 × 3			+	Chief	Died of pneumonia
35. Meyer	1917	36	M	1	RL			0.10 0.15 0.08 1.04	o	Wasserhelle generalized	
36. Bergstrand	1920	57	F	4							? renal rickets
37. Hubbard and Wentworth ...	1921	20	F	2		2 in diameter			+	Called hyperplasia	Others normal
38. Hartwich	1922	60	F	1	L	7 × 2.5 × 1	4.9		+	Chief (occasional wasserhelle)	

39. Nägelsbach and Westnes	1922	27	M	1		Pigeon's egg		+	+	No histology	Others normal
40. Günther	1922			1	RL			+	+	Chief	History lost
41. Fischer	1922	46	M	1	RL	$3.7 \times 2.7 \times 2$		+		No histology	
42. Strauch	1922	27	F	1	L	$4.8 \times 3.2 \times 3.5$? oxyphil (wasserhelle)	Puerperal osteomalacia
43. Sauer	1922	21	M	1	LL	Hazel nut		+	+	Chief	Calcified capsule
44. Bergstrand	1922	21	F	2	RL LL		0.31 0.32			? transition oxyphil	Uppers normal. Thy-mus enlarged
45. Bergstrand	1922	58	F	2	RL LL		0.12 0.37			? transition oxyphil	Uppers normal. Thy-mus enlarged
46. Pachner	1922	52	F	1	L	15×10				? transition wasserhelle	
47. Dawson and Struthers	1923	49	M	1	LL	2.5 in diameter		+		Chief	
48. Fasiani	1923	65	F	1		Fist				Chief (occasional wasserhelle)	
49. Stenholm	1924	24	M	1	RL	$1.8 \times 1.2 \times 1$		+	+	Chief	3 others normal
50. Stenholm	1924	52	F	1	RL	$3.3 \times 1.9 \times 1.1$		+	+	Glandular and cystic	3 others normal
51. Chauveau	1925	50	F	1		Hazel nut		+	+	Chief	
52. Gödel, Leb	1925	42	F	2	LL RL	10×2 8×1.8		+	+	Chief (? transition wasserhelle)	
53. Hoffheinz	1925	42		4	RL RU LL LU	$5.5 \times 3.4 \times 1.4$ $2 \times 0.5 \times 0.3$ $4.5 \times 2.1 \times 1.2$ $1.4 \times 1 \times 0.3$				Wasserhelle generalized	

TABLE VI (continued)

Author	Year	Age in yrs.	Sex	No. of tumors	Site	Size in cm.	Weight in gm.	Bone change	Renal stone	Cell type	Comment
54. Penecke	1926	38	F	1	LL		16	+	+	? transition oxyphil	Chronic nephritis
55. Penecke	1926	59	F	1	RL		5			Chief	Erysipelas
56. Mandl	1925 1926	38	M	1	LL	2.5 X 1.5 X 1.2		+		Transition wasserhelle	
57. Parreira and Castro Freire	1926	16	M	1	R	Chestnut		+		Wasserhelle generalized	
58. Hendrick	1926	71	F	1	Retro-sternal	8 X 5 X 4.5		+	+	Wasserhelle	
59. Wanke	1926	31	F	1	RL	Chestnut				Chief	
60. Gold	1928	54	F	1	RU	2.5 X 1.6		+	+	Chief (? transition wasserhelle)	Cerebral hemorrhage
61. Bergstrand	1928	48	F	4			Total 13.7			Glandular and cystic	
62. Beck	1928	41	F	2	RL LL	Coffee bean Hazel nut			+	No histology	
63. Eggers	1929		F	1	LL	Hazel nut			+	No histology	
64. Boyd et al.	1929	21	M	1	LL	3.5 X 2.5			+	Transition wasserhelle	
65. Barr et al.	1929	56	F	1	L	3 in diameter			+	Chief	
66. Barr et al.	1929	38	M	1		5 in diameter			+	Chief	

67. Guy	1929	29	F	1		8 × 6 × 4					Glandular and cystic	Recurred
68. Hunter	1929	41	F	1	LL	3.7 × 3 × 3		+			No histology	
69. Wellbrock	1929	32	F	1	Retro-sternal	5 × 3.5 × 3		+			Glandular and cystic	
70. Lloyd	1929	22	F	2	LL RL	1.7 × 1 × 0.8 1.5 × 0.4 × 0.3					Chief (occasional wasserhelle)	Pituitary tumor
71. Drennan	1930	63	F	1	RL	4.5 in diameter		+			Chief (occasional wasserhelle)	
72. Zajewloschin	1930	57	M	1	RL	4.3 × 3 × 1.3	8				Chief	Others normal
73. Pemberton	1930	14	F	1	LL	1.5 × 1.3 × 1.3		+			Chief	
74. Compere	1930	59	F	1	LL	1 × 1.8		+			Glandular and cystic	
75. Snapper	1930	56	M	1	LL	2.5 × 1.4		+			Wasserhelle generalized	
76. Rosenbach and Disqué	1930	24	F	1	RL			+			No histology	
77. Hecker	1930					Walnut		+			No histology	
78. Ask-Upmark	1930	46	M	1	L	1.5 × 1.2 × 1.2		+	0		Chief	
79. Léri <i>et al.</i>	1930	31	M	1	RL			+	+		Wasserhelle generalized	
80. Wanke	1930	38	F	1	RL	Walnut		+	+		Glandular	
81. Wanke	1930	41	F	2	RU RL	Coffee bean Almond		+	+		Chief	
82. Schupp	1931	51	F	1	In thyroid	1.5 × 0.5 × 0.8		+			Chief	3 others normal

TABLE VI (continued)

Author	Year	Age in yrs.	Sex	No. of tu- mors	Site	Size in cm.	Weight in gm.	Bone change	Renal stone	Cell type	Comment
83. Schouten	1931	55	M	1				+		No histology	
84. Silvestrini	1931	25	M	1	L	Olive		+		No histology	
85. Silvestrini	1931	57	M	1	L	1.5 X 2.5		+		No histology	
86. Allan	1931	34	M	1			10	+		No histology	
87. Snapper	1931	35	F	1	L	Almond		+		No histology	
88. Quick and Hunsberger, Schnabel	1931	25	M	1	Retro- sternal	5.2 X 3.5 X 2.5		+	+	Chief	Removed in two oper- ations
89. Paul	1931	56	M	2	RU LU	6 X 3 X 3 6 X 3 X 3		+	+	Wasserhelle generalized	Adrenals enlarged
90. Cosin	1931	17	M	1	RU	2.7 X 2.2 X 2		+	+	Transition wasserhelle	
91. Hunter and Turnbull	1931	49	F	2	LU RL	1.1 in diameter 2.8 X 1.8 X 2.5		+	+	Chief with giant forms	
92. Hunter and Turnbull	1931	37	F	2	RU LL	2.3 X 1.5 X 0.9 1.4 X 0.8 X 0.5		+	+	Wasserhelle generalized	
93. Hunter and Turnbull	1931	49	F	1	LL	6.8 X 2.8 X 1.4	13.5	+	+	? oxyphil ? wasserhelle	
94. Hunter and Turnbull	1931	51	F	2	RU RL	7.5 X 5 X 1.8 2 X 1.3 X 1.2	26.2	+	+	Called oxyphil	

95. Lièvre <i>et al.</i>	1931	41	F	I	RL	3.5 × 3 × 2		+		? transition oxyphil	
96. Cooley	1931	14	F	I				+		No histology	
97. Ask-Upmark	1931	43	F	I	L	4 × 2.2 × 1		+		Chief	
98. Bergstrand	1931	55	F	2	LU RL	4.5 × 2.5 × 3 4.5 × 2 × 0.5		+	+	Wasserhelle generalized	2 others slightly enlarged
99. Berner	1931	40	F	I	L	1.8 × 1.8	7	+		Wasserhelle focal	
100. Berner	1931	47	F	I	L	3 in diameter		+	+	Chief (occasional wasserhelle)	
101. Fraser	1931	42	F	I				+		No histology	
102. Fraser	1931	26	F	I				+	+	No histology	
103. Fraser	1931	23	F	I				+	+	No histology	
104. Weil	1931	44	F	I	RL	3.1 × 2.2 × 1.6	4.3	+	+	No histology	
105. von Redwitz	1931			I	RL	Cherry stone		+		No histology	
106. May and Lièvre	1931	45	M	I	LL	3 × 2 × 0.8	1.8	+	+	Chief	
107. Chievitz and Olsen	1932	25	F	I	RL	3.5 × 2 × 0.5		+		Chief	
108. Noble	1932	40	M	I	LU	2 × 1.8 × 1.5		+	+	Transition wasserhelle	
109. Hadfield and Rogers	1932	58	F	I	L	5 × 3.5 × 3	52	0		? wasserhelle generalized	Acromegaly
110. Hadfield and Rogers	1932	51	M	I		8 × 4 × 2.5			+	Chief	Acromegaly

TABLE VI (continued)

Author	Year	Age in yrs.	Sex	No. of tu- mors	Site	Size in cm.	Weight in gm.	Bone change	Renal stone	Cell type	Comment
111. Gaudier and Patoir	1932	60	M	1	RL			+		Glandular	
112. Beyerinck	1932	35	F	2	R and L				+	Called tubular adenoma	1 at operation 1 at autopsy
113. Frugoni and Alessandri	1932	18	F	1	R	2.2 X 1.1 X 0.6	0.9	+		Transition wasserhelle	
114. Rosedale	1932	50	F	1	R	2 X 2.5	7	+		Transition wasserhelle	
115. Mertz	1932	70	F	1	RL	1 X 1 X 0.5		+		Glandular and cystic	
116. Mertz	1932	64	F	1	RL	Normal		+		Chief	Calcium high Phosphorus low
117. Mertz	1932	73	F	1	RL	1 X 0.5 X 0.5		+		Chief	
118. Morelle	1932	56	F	1	LL	6 X 6.8 X 4.2	67	+	+	Chief	
119. Coryn	1932	20	F	1	In thy- roid	1.3 in diameter		+		Chief	3 operations to find tumor
120. Babcock	1932	25	F	1	LL	1.5 in diameter		+	+	No histology	
121. Wichmann	1932	45	F	1	LU	1.3 in diameter		+		Wasserhelle generalized	
122. Hellström	1932	42	F	1	LL	2 X 1.8 X 1.4		+		Chief	
123. Hellström	1932	44	F	2	R L	Walnut Walnut		+		Wasserhelle generalized	Removed at 3 months interval

124. Hanke	1932	33	F	2	R L	3.1 × 3 × 1.2 Hazel nut		+	+	Called oxyphil	2 others slightly enlarged
125. Hanke	1932	49	F	2	RL LL	5 × 3 × 2 Walnut		+	+	Wasserhelle generalized	
126. Gordon-Taylor ..	1932	20	F	2	R L	3.5 × 2.5 × 2.5 Enlarged		+		Wasserhelle generalized	
127. Wilder <i>et al.</i>	1932	48	F	1	RU	2 × 1.5 × 1		+		Wasserhelle generalized	3 others slightly enlarged. Same histology
128. Rusakov and Sakayan	1932	50	F	2	L R	Cherry stone 3 × 2 × 1		+	+	Chief (occasional wasserhelle)	
129. Renaud <i>et al.</i>	1932			1	Retro- sternal	4 × 2.5		+		Chief (occasional wasserhelle)	
130. Cohen and Kelly	1933	48	F	1	RL	2.5 × 1.5 × 1	2.5	+	+	Chief	
131. Elmslie <i>et al.</i>	1933	42	F	1	LL	3 × 1.5 × 1.5		+	0	Transition wasserhelle	
132. Elmslie <i>et al.</i>	1933	26	F	1	R	2.5 × 2 × 2		+	+	Transition wasserhelle	
133. Elmslie <i>et al.</i>	1933	23	F	1	L	3.5 × 1.5 × 0.7		+	+	Chief	
134. Thomason and Smith	1933	41	F	1	LU	2.2 in diameter		+		Chief	
135. Struthers	1933	38	F	1	R	1.8 in diameter		+		? Chief	
136. Struthers	1933	48	F	1		1.8 in diameter		+	+	No histology	

TABLE VI (continued)

Author	Year	Age in yrs.	Sex	No. of tu- mors	Site	Size in cm.	Weight in gm.	Bone change	Renal stone	Cell type	Comment
137. Copello and Barlaro	1933	46	M	1	LL	1.8 × 1.8 × 1.2		+	+	Called adenoma	
138. Venables	1933	52	F	1	In thy- roid			+		Called oxyphil	
139. Rankin and Priestly	1933	34	F	1	R	6 in diameter		+		Chief with giant forms	
140. Schlesinger and Gold	1933	42	F	1	In thy- roid	5 × 4 × 4		+	+	Glandular and cystic	Calcium high after a 1st operation. LU found at 2nd operation
141. Sainton <i>et al.</i>	1933	43	M	2	LL LU	Enlarged		2.1	+	? oxyphil ? wasserhelle generalized	
142. Keynes and Taylor	1933	18	M	1	LL	3.5 × 2.5			+	Chief	
143. Dyke <i>et al.</i>	1933	14	F	1	LL	3 × 2 × 1			+	Transition wasserhelle	
144. Dyke <i>et al.</i>	1933	14	F	1	RL			5	+	Transition wasserhelle	
145. Abel <i>et al.</i>	1933	58	F	1	LL	4 × 1.8 × 1.6		4.3	+	Chief with ? giant forms	
146. Mandl	1933	47	F	1	Retro- sternal	4 × 2.5 × 2			+	Chief	Pylonephritis
147. Hand	1933	39	M	1	LL	4 × 2.5 × 2			+	Glandular and cystic	

148. Morton	1933	20	F	1	RL	$2.1 \times 1.8 \times 0.5$	1.3	+	+	Transition wasserhelle	Other glands negative
149. Schwensen and Eiken	1933	36	F	1		$5 \times 3.5 \times 2$	37	+	+	Chief	
150. Khurgina	1933	34	M	1	LL	2.5×1		+	+	Chief	Diffuse calcium deposits
151. Mimpriss and Butler	1934	17	M	1	Retro-sternal	1.2 in diameter		+		Chief (occasional wasserhelle)	
152. Barker	1934	65	F	1	RL	$2.5 \times 2 \times 1$		+	+	No histology	
153. Strandgaard	1934	47	F	1	R	1×1.7	2	+	+	Transition wasserhelle	
154. Sørensen	1934	74	M	1	LL	$1.5 \times 3.5 \times 2.5$	7	+		Chief	
155. Capps	1934	50	M	2	RL LL	$7 \times 3.5 \times 2.1$ $2.4 \times 3.5 \times 2.1$	22 13.6			Wasserhelle generalized	
156. Gutman <i>et al.</i>	1934	34	M	1	RL	$2.7 \times 1.6 \times 1$	3.5	+	? +	Chief	
157. Gutman <i>et al.</i>	1934	53	F	1	RL	$3 \times 2 \times 0.8$	4.5	+	+	Wasserhelle generalized	
158. Gutman <i>et al.</i>	1934	35	F	1				+		No histology	
159. Gutman <i>et al.</i>	1934	60	F	2	RU RL	2 in diameter $1.5 \times 0.7 \times 0.4$		+		Called oxyphil	
160. Bergstrand	1934	64	F	1	In thy-mus	Pigeon egg		+	+	Chief	4 others normal, adrenals enlarged, metastasizing thyroid adenoma

cell hyperplasias prove that a physiological stimulus can convert every parathyroid cell into the wasserhelle type.

The neoplasias of the glands serve to reinforce the arguments against generically different cell types and favor the concept of a fundamental cell from which all others are derived. Pure tumors of either the oxyphil or wasserhelle type unaccompanied by any chief cell forms were not present in our series and we find the occasional reports in the literature unconvincing. When cells of either of these specialized types are predominant numerous transition forms of the chief cell can always be demonstrated. The chief cell in other words is the only invariable component of a tumor, obviously the basic fundamental cell and possibly the only proliferative form. The other cell types derived from it are to be regarded as degrees of differentiation or as involution forms.

SUMMARY

The histology of the parathyroid glands in 25 cases of hyperparathyroidism has been reported in detail and contrasted with the normal glands removed from 150 routine autopsies. It was found possible to divide the cases sharply into two groups, one of them characterized by diffuse uniform changes throughout all the glandular tissue—an obvious hyperplastic process—the second by a proliferative area limited to one gland, frequently even to a portion of it, or rarely involving parts of two glands. For reasons which have been discussed at length in the text, we regard this localized type of growth as neoplastic.

On this basis a classification of the parathyroid changes in hyperparathyroidism into two primary groups, hyperplasia and neoplasia, with subgroups under each heading, based on the morphological criteria of predominant cell type and structure, has been proposed. It has been shown that this is applicable not only to our own series but to the entire group of 160 cases which we have been able to collect from the literature.

An effort has been made to compile adequate statistical data on the relative frequency of these types of hyperparathyroidism and also on age and sex incidence, the frequency of multiple growths, the location of the tumors and the relation of both types of the disease to osteitis fibrosa cystica and to renal stone formation. A rough quan-

titative relation between the size of the enlarged glands and the degree of hyperfunction has been demonstrated.

Finally, an attempt has been made to bring to bear such data as a study of parathyroid tumors affords upon the problems of the function and the histogenesis of the various cell types.

CONCLUSIONS

1. The pathological findings in the parathyroid glands in hyperparathyroidism may be divided sharply into two types, hyperplasia and neoplasia.

2. Hyperplasia is characterized by diffuse uniform involvement of all the glandular tissue. It occurs, however, in two forms, a wasserhelle or water-clear cell type, and a much rarer chief cell type.

3. Localized tumors of a single gland, part of a gland, or rarely parts of two glands, are more logically to be regarded as neoplasms.

4. A roughly quantitative relation between the size of the enlarged glands and the degree of hyperfunction exists.

5. The histology of parathyroid tumors provides confirmatory evidence for the monophyletic theory of origin of the various cell types.

6. Glycogen, albeit in minute amounts, is always present in functioning parathyroid tissue and the concept of the oxyphil cell as an inactive involution product receives support from a study of the adenomas.

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DESCRIPTION OF PLATES

PLATE I

- FIG. 1. A longitudinal section of a whole parathyroid gland from a child 16 months old, showing the compact grouping of the chief cells and the absence of fat. $\times 20$.
- FIG. 2. A longitudinal section of a whole normal parathyroid gland from an adult 40 years of age, showing the relative proportions of parenchymal and fat cells. $\times 15$.
- FIG. 3. A longitudinal section of a whole normal parathyroid gland from an adult 80 years of age, showing numerous circumscribed islands of pale oxyphil cells. $\times 15$.
- FIG. 4. A longitudinal section of a whole parathyroid gland showing a large cyst and two minute encapsulated adenomas. $\times 12$.
- FIG. 5. A longitudinal section of a whole parathyroid gland showing an apparently non-functioning, well circumscribed adenoma. Fat cells which are in normal numbers in the surrounding gland are nearly absent in the tumor. $\times 12$.



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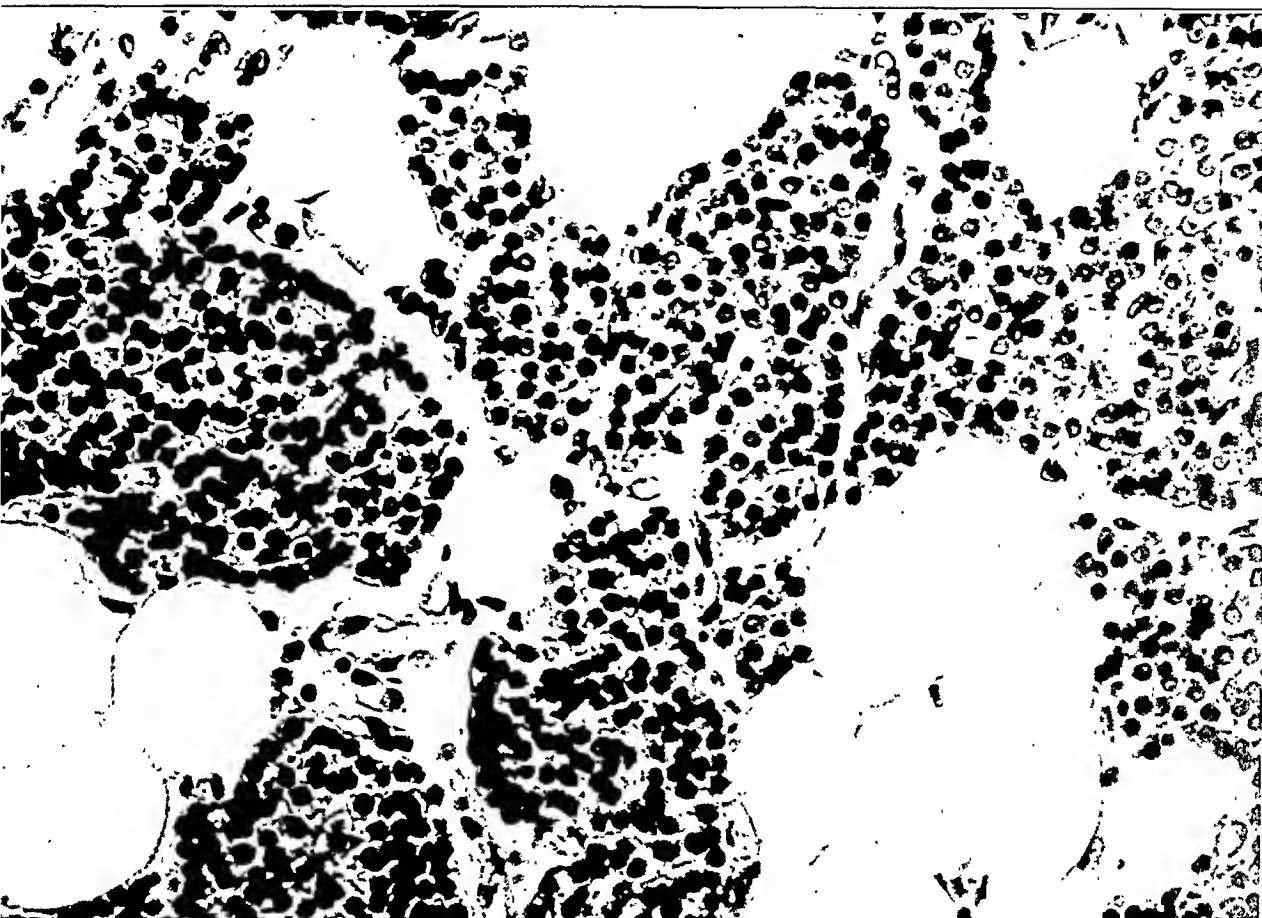


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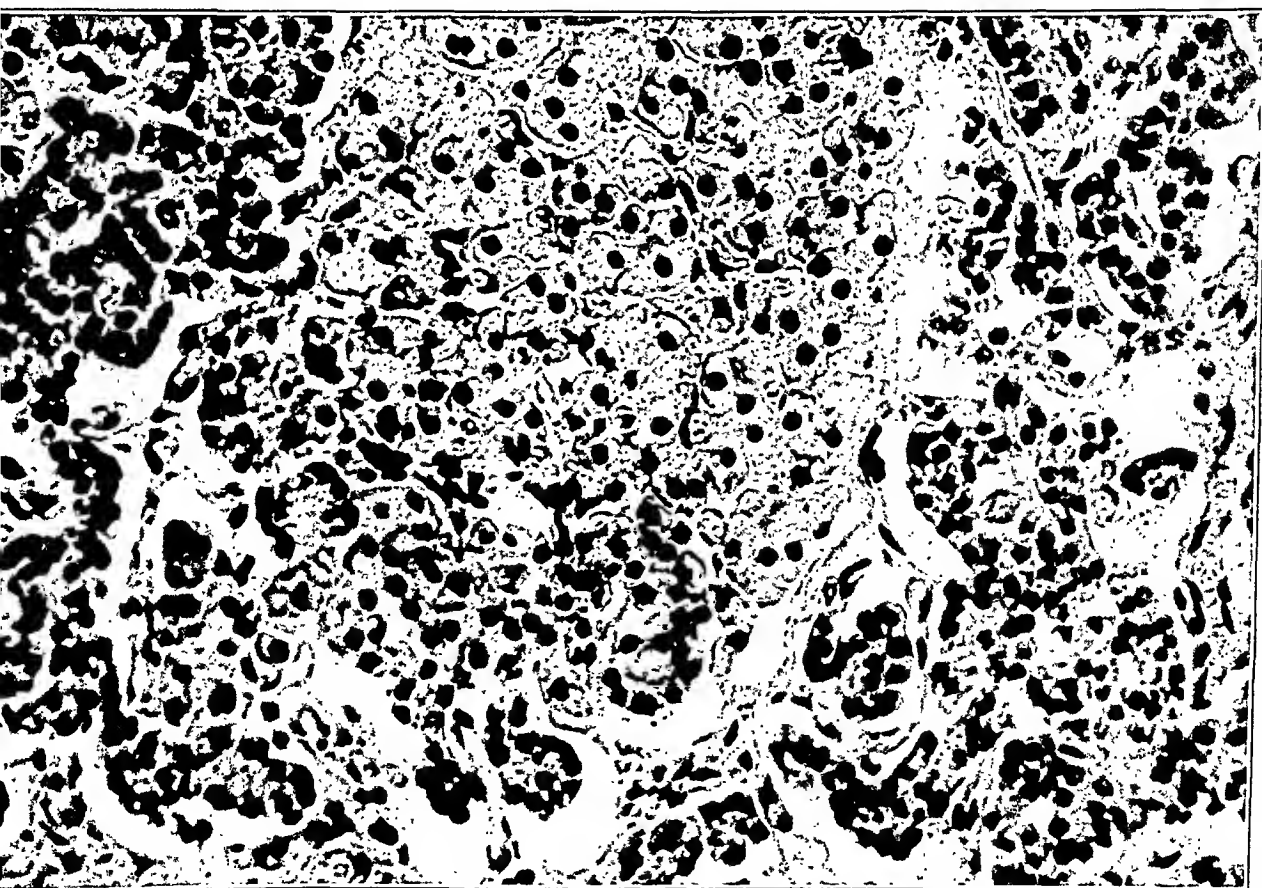
PLATE 2

FIG. 6. A higher magnification of a small portion of the gland shown in Fig. 2, showing the normal chief and large fat cells. $\times 400$.

FIG. 7. A higher magnification of an island of pale oxyphil cells from the gland shown in Fig. 3. Note the surrounding normal chief cells and the lack of extracellular fat globules. $\times 400$.



6



7

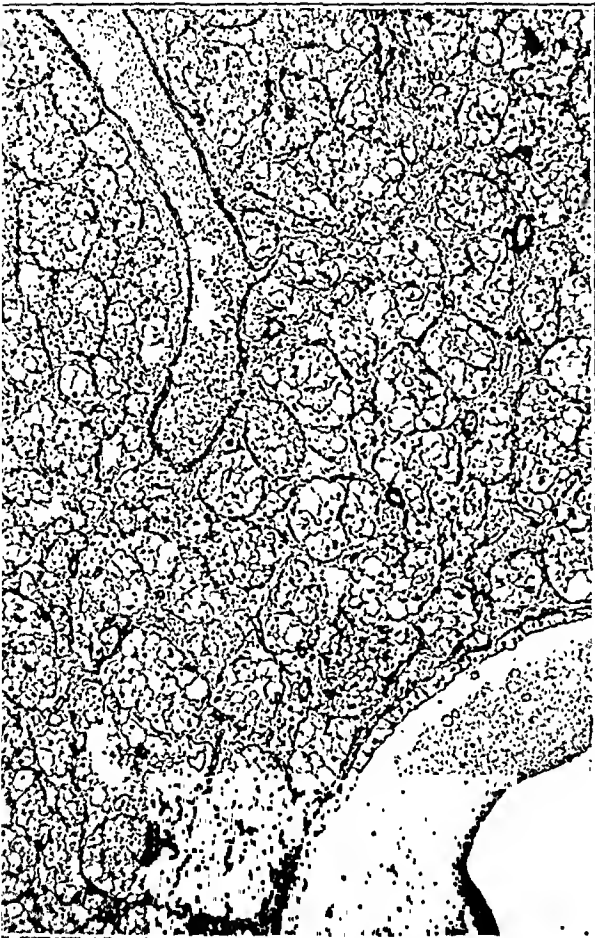
PLATE 3

FIG. 8. Case 15. A low power view of clear cell hyperplasia. The marked uniform acinar arrangement, the swollen clear cells, the abundant vascular stroma and the absent fat are characteristic. One huge cyst is present. $\times 50$.

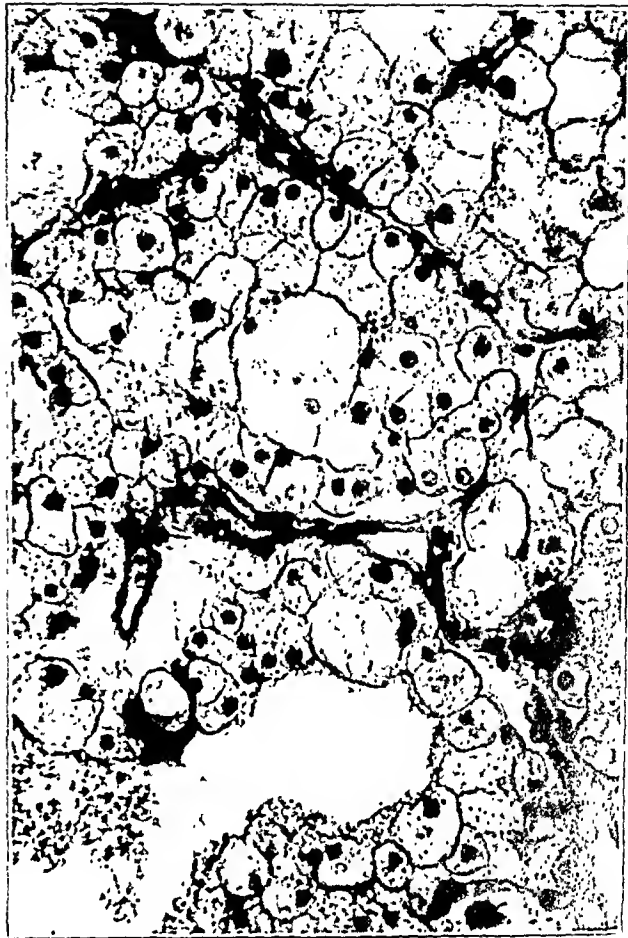
FIG. 9. A higher power of Fig. 8. Note the definite gland formation, the sharply outlined large epithelial cells, the cytoplasm absent except for scattered granules, and the dark basally oriented nuclei. $\times 400$.

FIG. 10. Case 16. The typical pattern in a case of parathyroid hyperplasia produced by the basal orientation of the nuclei. $\times 50$.

FIG. 11. A higher power of Fig. 10. The nuclei of many of the cells lie out of the plane of section. When present, they are hyperchromatic and clearly oriented toward the stroma. $\times 400$.



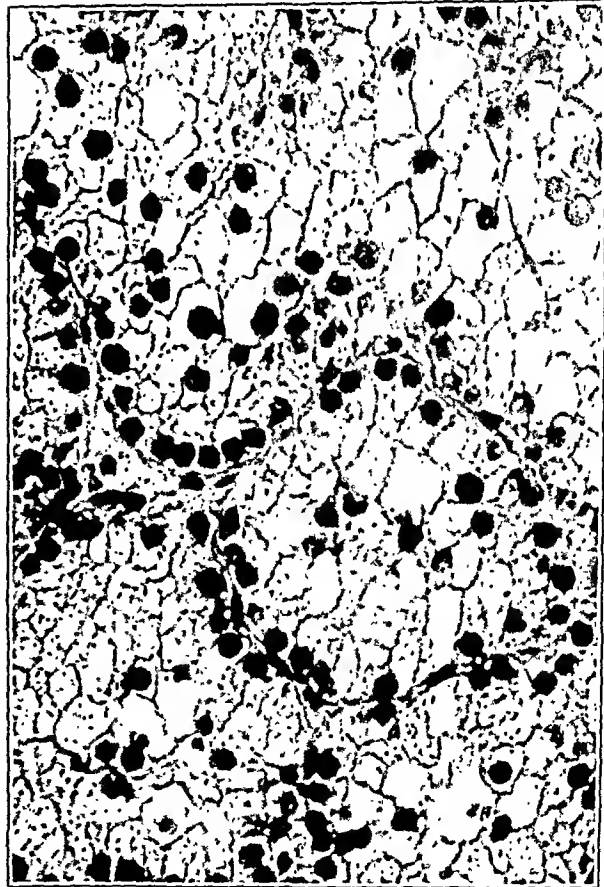
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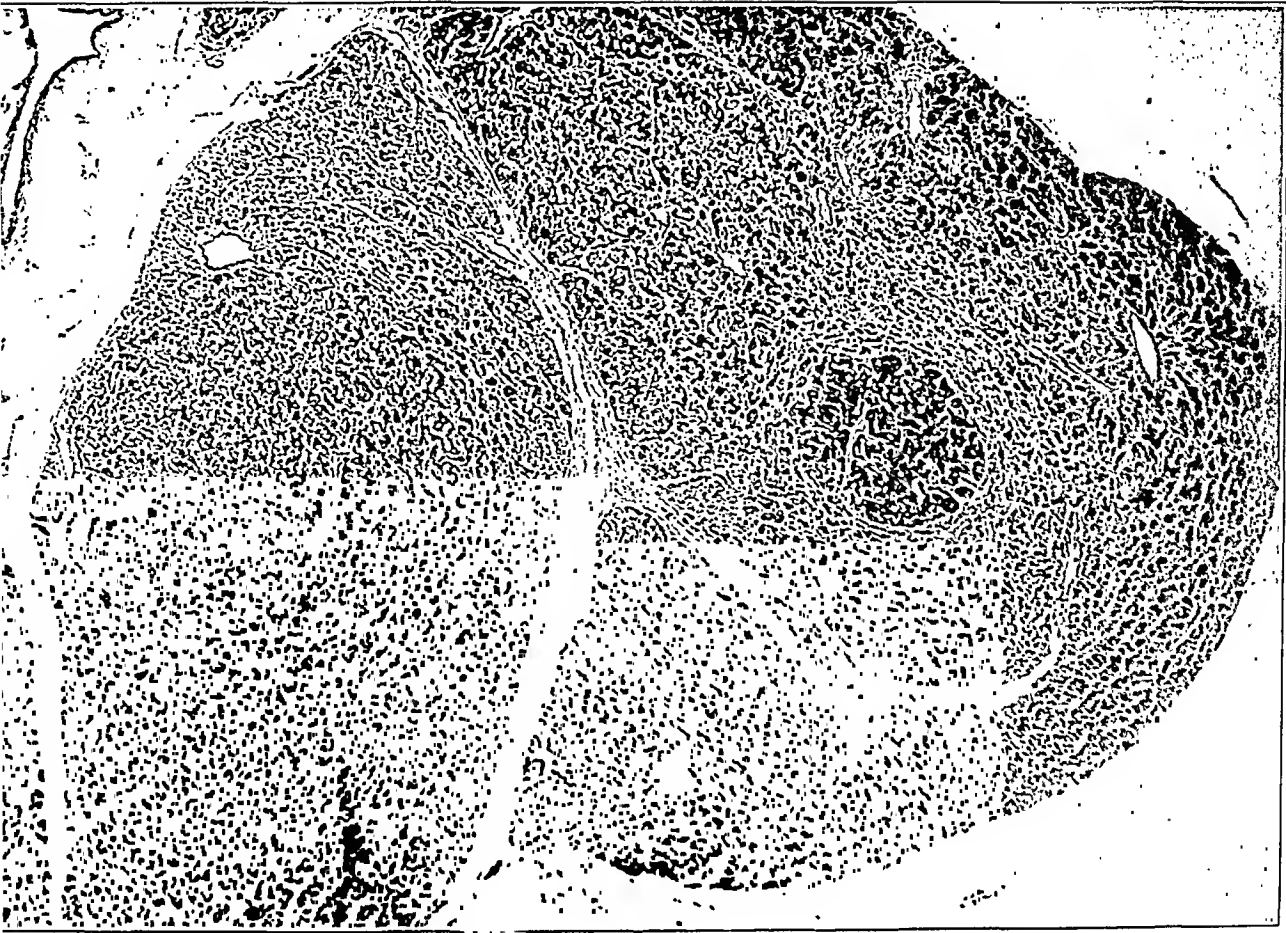


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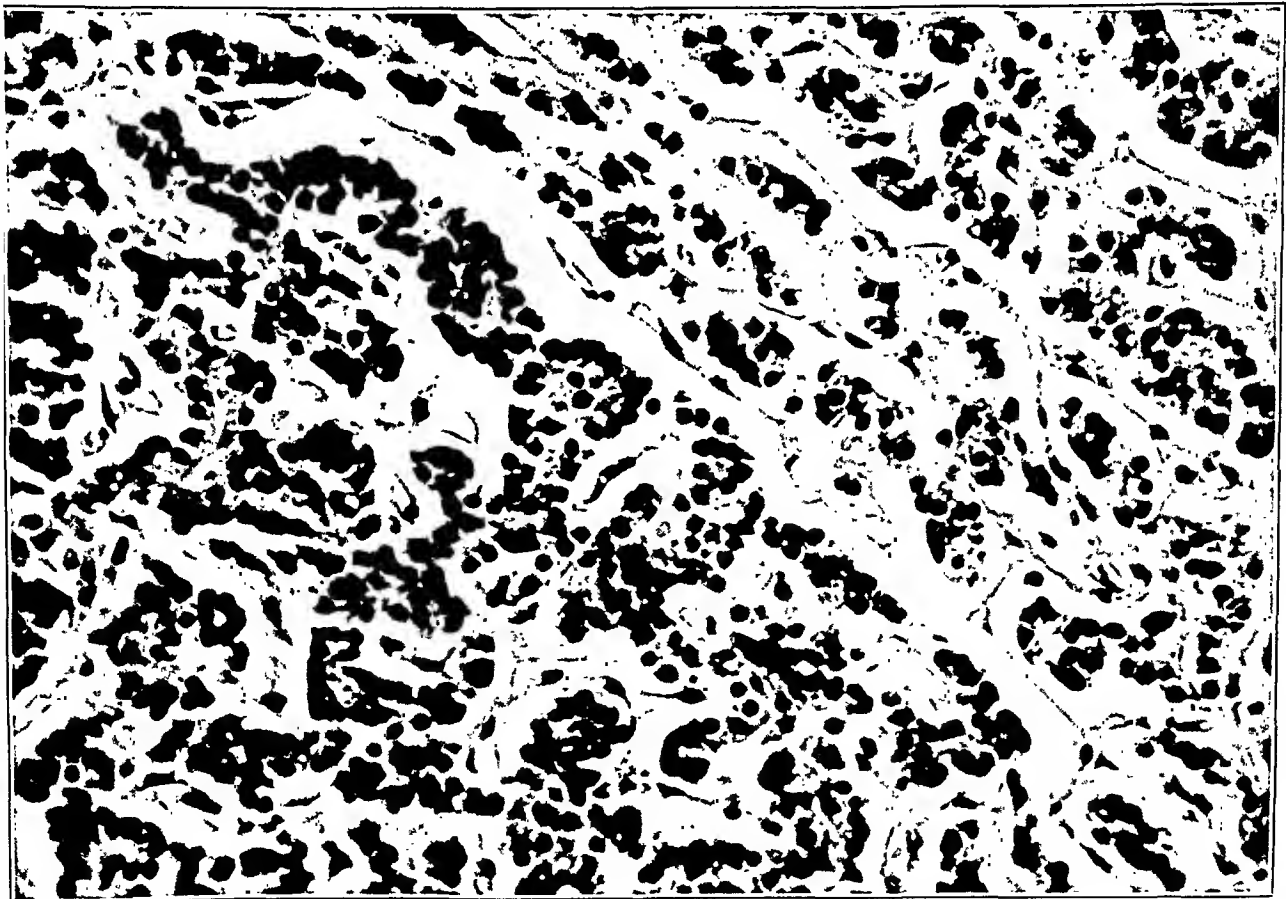
PLATE 4

FIG. 12. Case 23A. A case of parathyroid hyperplasia of the chief cell type. Note the compactness of the cell arrangement, the absence of intercellular fat and the papillary acinar arrangement in one area. $\times 30$.

FIG. 13. A higher power of Fig. 12 taken at the edge of the large circumscribed papillary area, showing the marked basophilism in contrast to the surrounding tissue. $\times 400$.



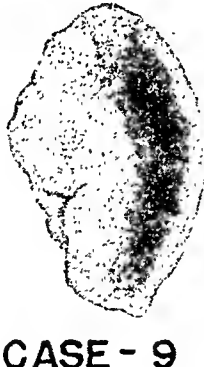
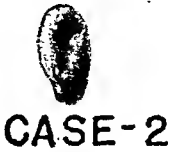
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PLATE 5

FIG. 14. An actual size drawing of the tumors removed from Cases 1 to 11. Note the variability in size and in shape.

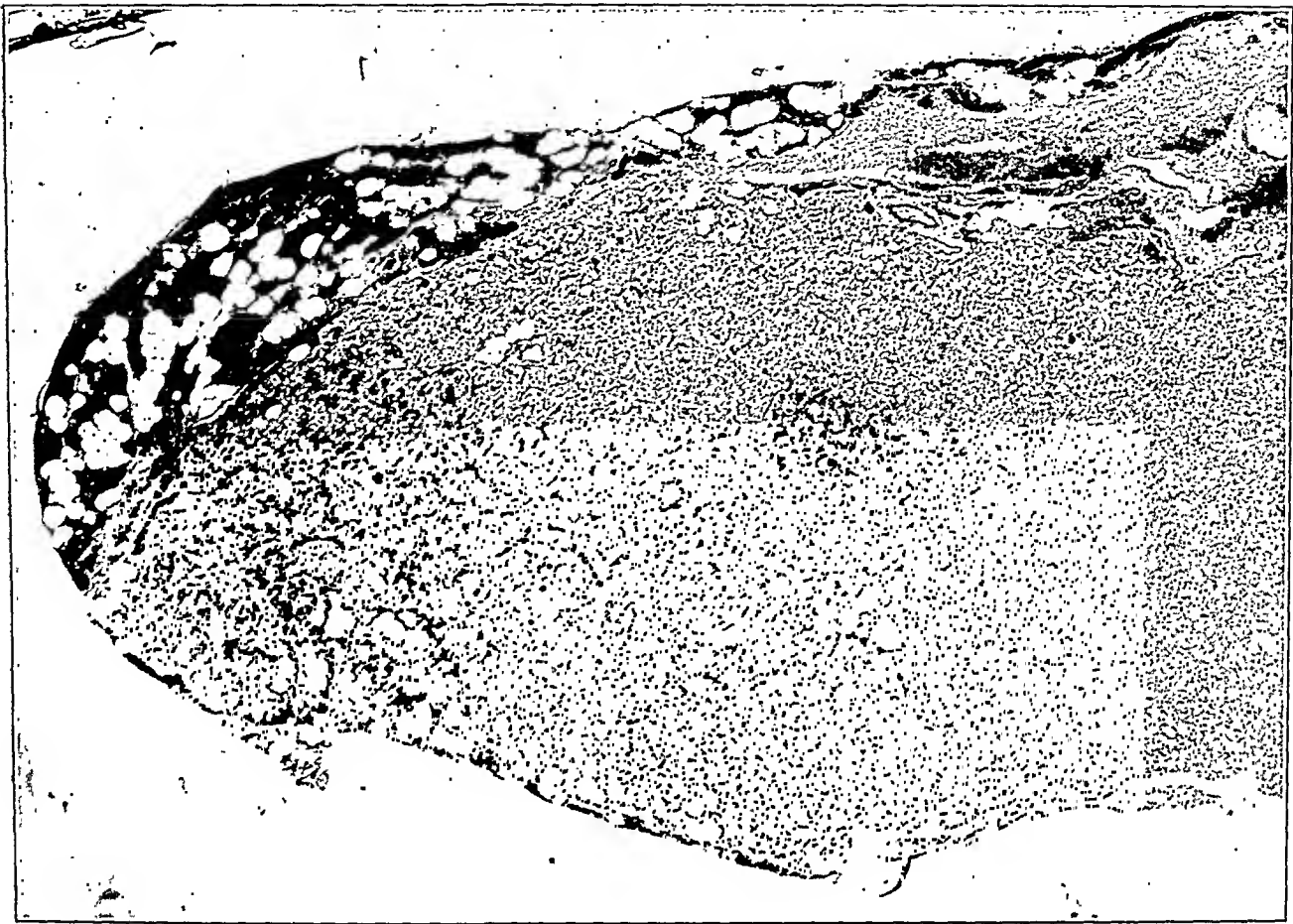


E. B. Hedges '33

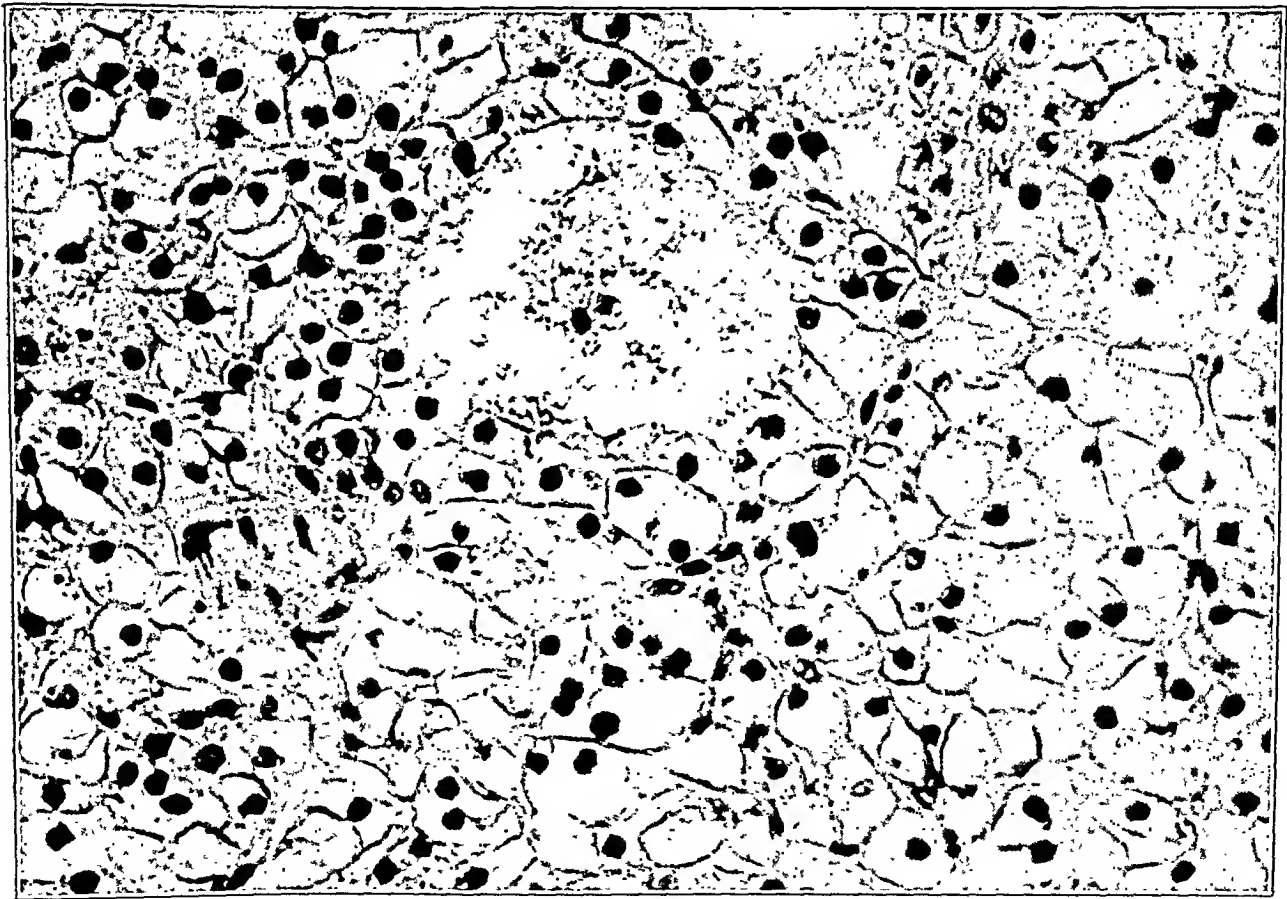
PLATE 6

FIG. 15. Case 2. A longitudinal section through almost the entire tumor showing a rim of normal parathyroid tissue surrounding a wasserhelle adenoma. In the latter is a localized group of transition oxyphil cells. $\times 15$.

FIG. 16. A higher power of Fig. 15, showing the sharply demarcated wasserhelle cell. $\times 400$.



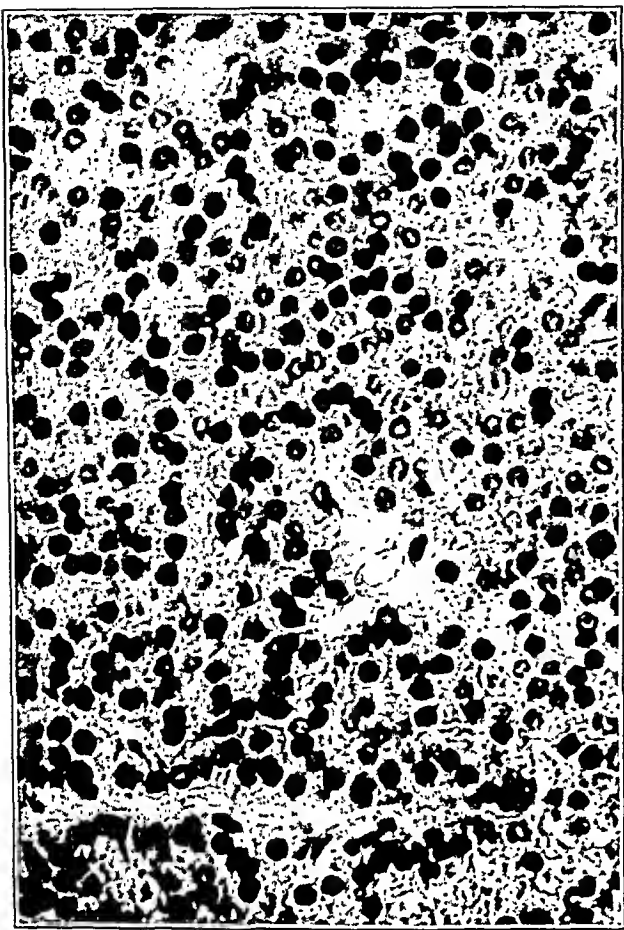
15



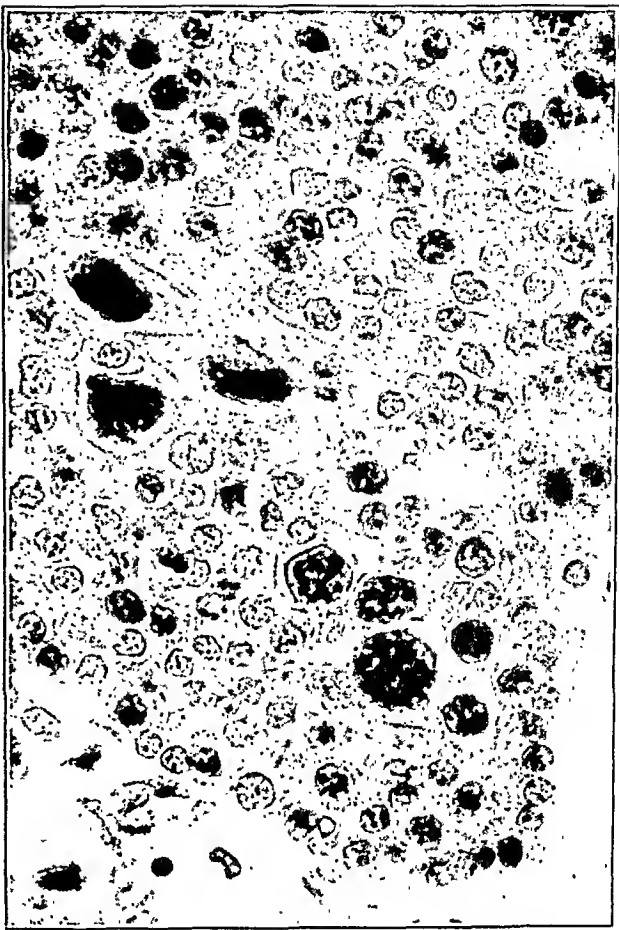
16

PLATE 7

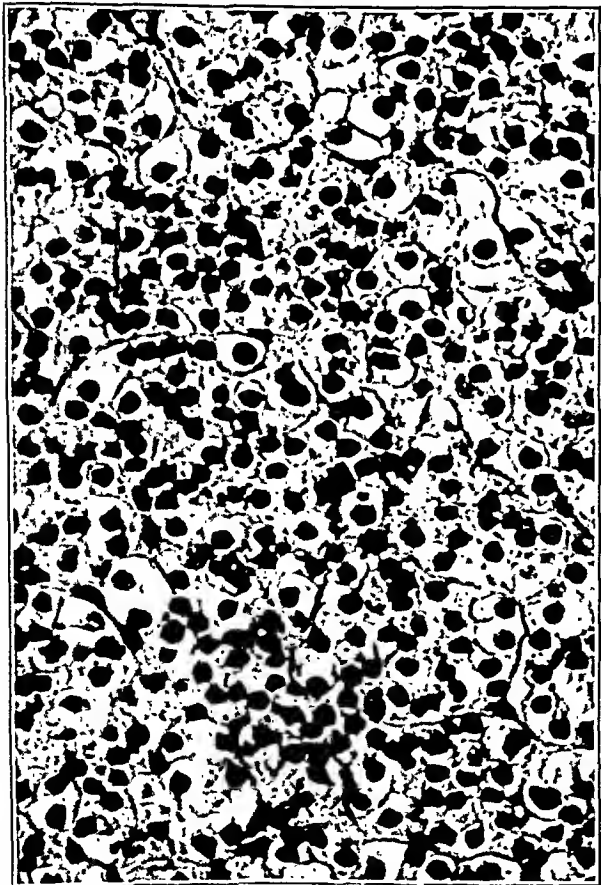
- FIG. 17. Case 6. An example of a chief cell tumor showing the enlarged chief cell with its poorly outlined cell margin, its large hyperchromatic nucleus and its faintly acidophilic cytoplasm. Note the increased vascularity, the compact grouping of the cells and the absence of fat. $\times 400$.
- FIG. 18. Case 11. A chief cell tumor with numerous greatly enlarged cells and giant hyperchromatic nuclei. Even the smaller cells are well above the normal in size. $\times 400$.
- FIG. 19. Case 3. An example of a transition wasserhelle cell tumor. About the nuclei clear halos of varying width can be seen. Occasionally they extend to the cell margins. The cells closely contiguous to the stroma are barely discernible. $\times 400$.
- FIG. 20. Case 19. An example of a transition oxyphil cell tumor. These cells show transition stages from the chief to the pale oxyphil cell. Note the granular abundant cytoplasm and the pseudoglandular arrangement. $\times 400$.



17



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19



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PLATE 8

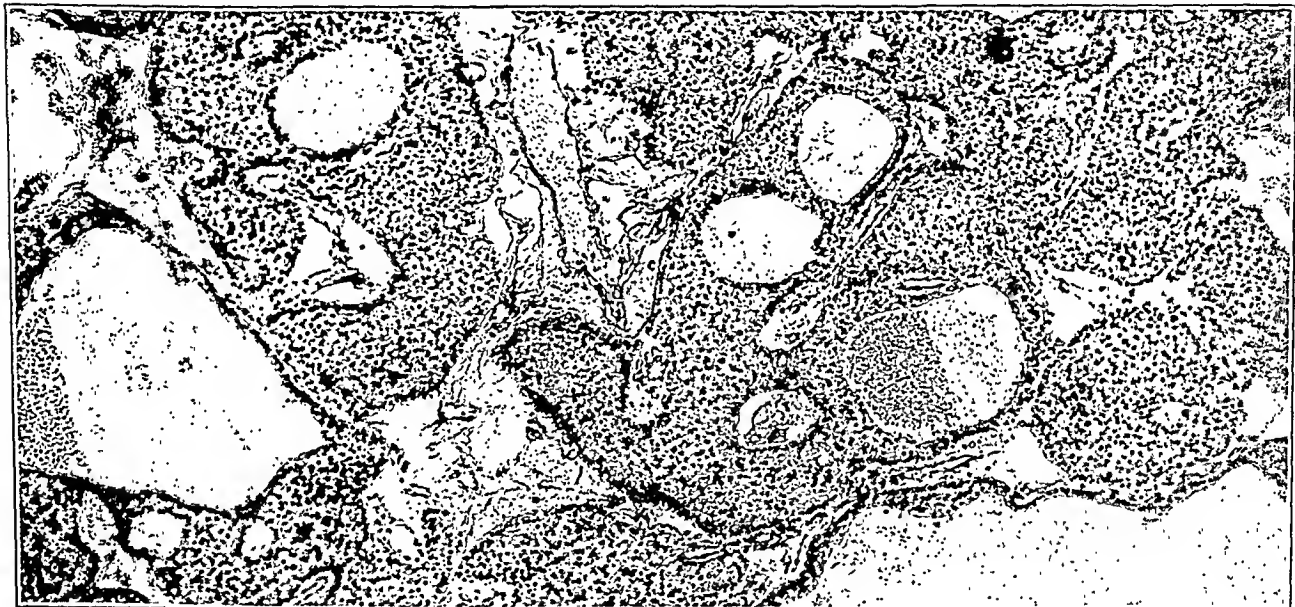
FIG. 21. Case 4. An example of a glandular and cystic chief cell tumor. In addition to the glandular area in the upper part of the photomicrograph note the pale oxyphil cells on the left and the chief cells below. $\times 50$.

FIG. 22. Case 13. Another example of the glandular and cystic type. In this case the spaces are not so close to each other and are larger. Note the presence of red blood cells in some of the glands. $\times 50$.

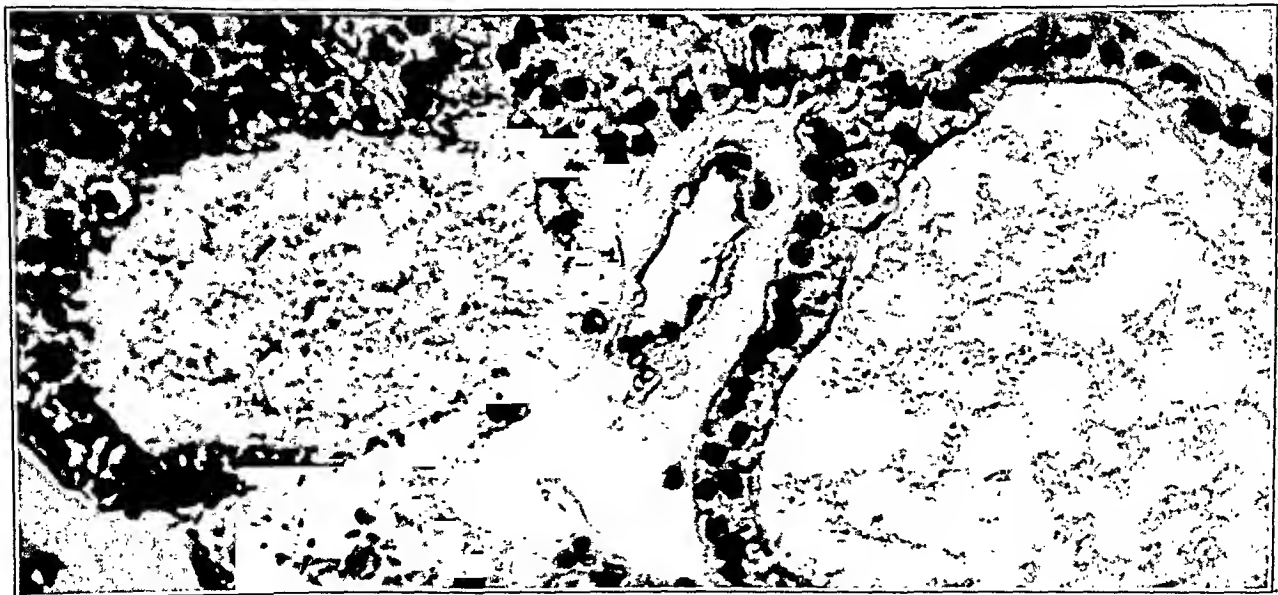
FIG. 23. Case 13. A higher power of Fig. 22, showing the chief cells lining these spaces. $\times 400$.



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22

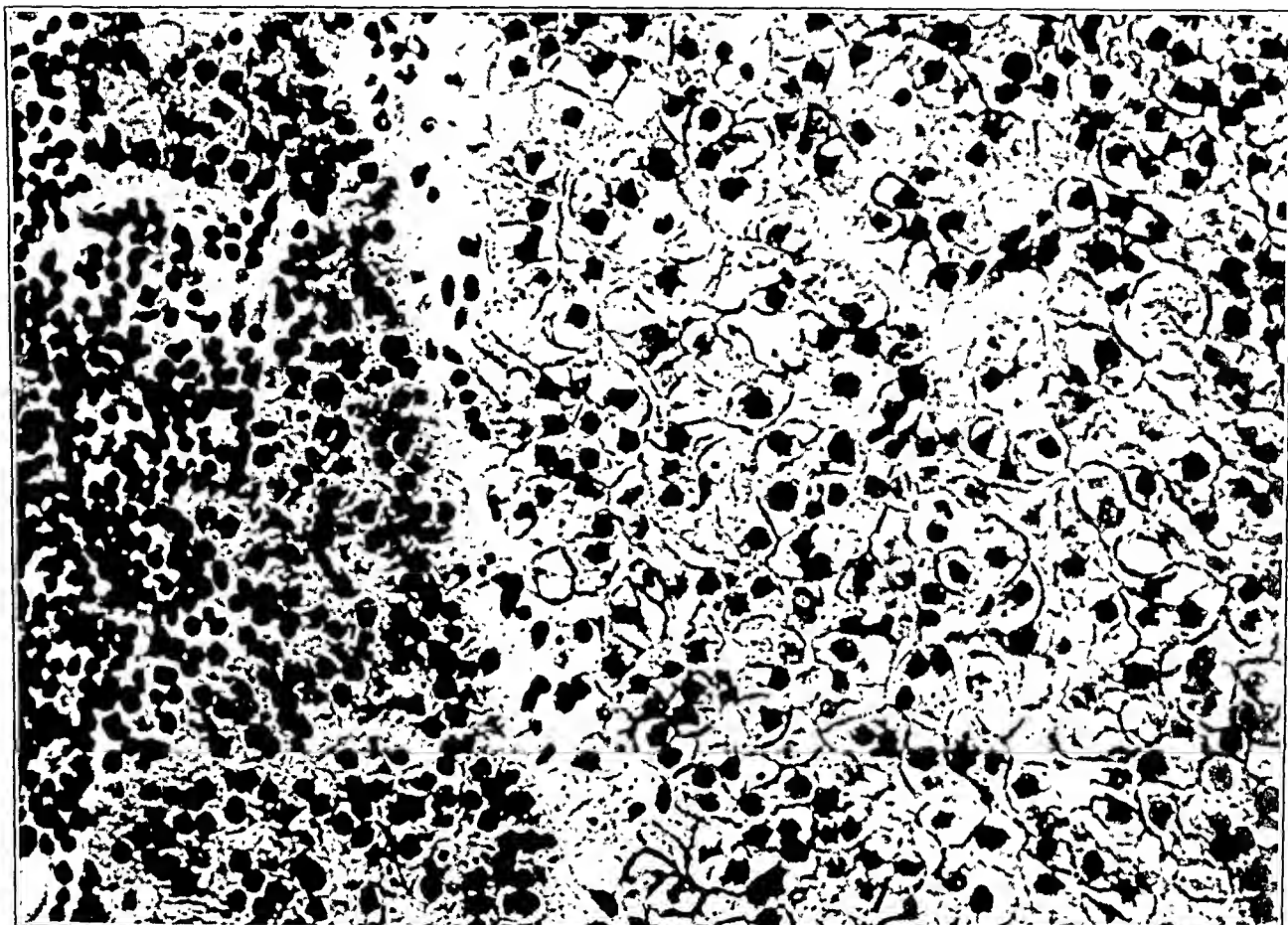


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PLATE 9

FIG. 24. An example of a focal wasserhelle cell tumor showing the large islands of waterclear cells, surrounded by moderately enlarged chief cells. $\times 400$.

FIGS. 25 and 26. Case 20. An example of multiple chief cell tumors showing the dissimilarity of two tumors in the same case. One is definitely glandular; the other belongs to the transition wasserhelle cell type and is non-glandular. $\times 100$.

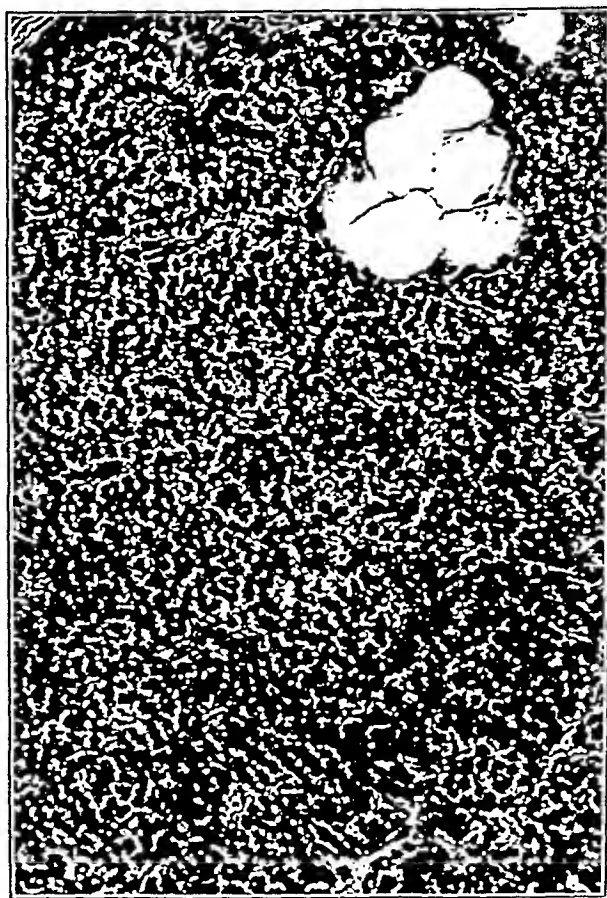


24



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Castleman and Mallory



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Pathology of Parathyroid Gland

ENLARGEMENT OF THE PARATHYROID GLANDS IN RENAL DISEASE*

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The initial impetus to this study was given by a case of typical osteitis fibrosa with adenomatous enlargement of three parathyroid glands and associated polycystic kidneys.† The question which arose in the discussion was in regard to the relation between the kidney disease, obviously congenital in origin, and the parathyroid enlargement.

Since the report by MacCallum,¹ in 1905, of parathyroid adenoma associated with chronic glomerulonephritis, the simultaneous occurrence of renal lesions with parathyroid tumors or enlargement has been noted repeatedly. This association has been emphasized particularly in the recent study of Albright, Baird, Cope and Bloomberg,² who collected 83 cases of hyperparathyroidism, 43 of which showed some type of renal damage. The renal lesions are attributed to the precipitation of calcium in the tubules, with resultant sclerosis, contraction and insufficiency, or to the formation of calculi in the pelvis associated with pyelonephritis.

Thus, these authors in general regard the renal lesions as a sequel to the chronic hyperparathyroidism and stress especially the deposition of calcium in the renal tissue as the proximate cause of the kidney lesions. However, in their discussion they raise the question as to whether the parathyroid enlargement may not be secondary to the renal disease in the cases in which multiple glands are affected. They report: "It seems conceivable that a chronic renal insufficiency with phosphate retention and a high inorganic phosphorus level might likewise cause hyperplasia of all parathyroid tissue which might go on to multiple tumor formation. . . . In these cases, the kidney damage may be the cause and not the result of the parathyroid tumors."

* Received for publication July 26, 1934.

† This case has been reported in detail by Gutman, A. B., Swenson, Paul C., and Parsons, W. B. The differential diagnosis of hyperparathyroidism. *J.A.M.A.*, 1934, 103, 87 (Case 4, page 90).

Bergstrand³ in a routine study of the parathyroids in 200 autopsies found a small percentage in which the glands were "distinctly enlarged" and in most of these cases there were at the same time more or less severe changes in the kidneys. Subsequently, a series of nephritis cases was studied: in 10 of 50 cases the combined parathyroid weights exceeded 200 mg., which he regards as the upper limit of normal. We shall refer to these findings again after an analysis of our own data.

Vines⁴ in his monograph states: "In chronic nephritis a somewhat similar hyperplasia (*i.e.*, as that in rickets) has been found." He gives no data or references, however.

With this suspicion of a relation between renal disease and parathyroid enlargement before us, it seemed worth while to begin a systematic study, in order to determine whether disease of the kidneys might not lead quite regularly to enlargement of the parathyroids.

MATERIAL AND METHODS

The parathyroid glands in a series of 27 nephritic and 72 miscellaneous cases were dissected out and weighed individually on a Roller-Smith torsion balance, sensitive to 0.2 mg. They were then fixed in Zenker's fluid and sectioned serially for identification and microscopic study. A second series of 29 nephritic and 12 control cases was obtained from neck organs which had been preserved in Klotz or Kaiserling fluid. These glands were also weighed and sectioned. The weights of these fixed specimens were found to be somewhat less than that of the fresh material, but no constant variation was found. The data obtained from this material will therefore be presented separately.

WEIGHTS OF PARATHYROIDS IN NON-NEPHRITIC CASES

In order to have a reliable standard of comparison it was necessary to obtain data on a sufficient number of control cases to show the range of biological variation in comparable groups of individuals. The ideal data for this purpose, as Bergstrand has pointed out, would include only glands from healthy persons who had died suddenly from accidental causes. Such material was not available, and we have been forced to take as controls the weights of glands from non-nephritic patients dying of various diseases. Knowing nothing of the effect which such diseases might have upon the parathyroids, it cannot be assumed that these weights represent "normal" values in a strict sense, but for the purpose of our inquiry, namely to ascertain if nephritis is commonly associated with parathyroid enlargement,

they would seem to provide a sufficiently accurate standard of comparison. So, also, a calculation of the amount of functional parenchyma would have to take into account the relative amount of interstitial fat and fibrous tissue. No attempt has been made to correct for this variable, since there seems to be no simple method for so doing, and in the comparison of fairly large numbers the error introduced by neglecting this would not appear to be significant.

The literature contains surprisingly few systematic studies of the weights of the human parathyroid. The available references to parathyroid weights in adults are given in tabular form (Table I).

TABLE I
Weights of Parathyroid Glands Cited from Literature

Author	Year	Mean weight		Combined weight
		Upper	Lower	
Welch ⁵	1898	gm. 0.035		gm.
Marañón ⁶	1911	0.020-0.050		0.080-0.120
Danisch ⁷	1924	0.026-0.030	0.037-0.041	
Marine ⁸	1928	0.020	0.035	
Aibara ⁹	1931			0.067

Bergstrand,³ 1921, gives as the upper limit of normal combined weight 200 mg., a figure which on the basis of our own data seems considerably in excess of normal. The weights which we have obtained in our series of non-nephritic control cases in which the kidneys showed no microscopic lesions of significance are presented in Table II.

These data are summarized in Table III, which gives also the standard deviation and the probable error of the mean. These figures are in close agreement with those of Danisch.

INFLUENCE OF SEX ON THE WEIGHT OF NORMAL PARATHYROID GLANDS

It is evident from the table that the mean weights of each gland in the female exceeds that of the male. In the case of the right upper, right lower, and left upper, the difference is statistically significant.

TABLE II

Weights of Parathyroid Glands in Cases with Normal Kidneys

Principal diagnosis

Autopsy No.	Age yrs.	Sex	Right		Left		Combined weights gm.	Principal diagnosis
			upper gm.	lower gm.	upper gm.	lower gm.		
11,392	54	F	0.035	0.058	0.026	0.031	0.123	Bacteremia, hemolytic staphylococcus
11,393	31	F		0.032	0.025			Chorionic carcinoma
11,394	42	F		0.014				Glioblastoma
11,402	56	M	0.024	0.030	0.016	0.069	0.163	Carcinoma of urethra
11,403	64	M		0.094				Carcinoma of urethra
11,404	51	M		0.025				Lobar pneumonia
11,405	55	M						Lobar pneumonia
11,406	29	F	0.026	0.019	0.019	0.027		Bacteremia, hemolytic staphylococcus
11,408	58	F	0.016	0.059	0.038	0.031		Epithelioma of uterus
11,410	60	M						Bacteremia, hemolytic staphylococcus
11,411	41	F	0.036		0.029	0.041	0.075	Epithelioma of uterus
11,413	41	M	0.036		0.041	0.016		Arteriosclerosis
11,415	50	F	0.022		0.041	0.037		Arteriosclerosis
11,419	25	F	0.025		0.035			Lobar pneumonia
11,423	50	F	0.048		0.018			Emphysema
11,425	74	F	0.017	0.047	0.018	0.027	0.093	Septic aneurysm of aorta
11,427	58	F	0.029	0.031	0.038	0.034	0.167	Syphilitic aneurysm of pharynx
11,431	47	F	0.026	0.066	0.038	0.030		Lymphoepithelioma of pharynx
11,435	40	M	0.039	0.029				Aneurysm of abdominal aorta
11,436	26	F	0.017	0.022	0.037	0.040	0.173	Abscess of brain
11,438	64	M	0.047	0.036	0.050			Aneurysm of cerebral artery
11,440	44	M	0.062	0.058	0.031			Abscess of stomach
11,441	67	F			0.006			Carcinoma of stomach
11,447	42	M	0.012					Rheumatic heart disease
11,449	74	M	0.013	0.016	0.034	0.031	0.118	Arteriosclerosis
11,450	14	M	0.011	0.012	0.020	0.034	0.107	Rheumatic heart disease
11,460	55	M	0.026	0.027	0.024			Carcinoma of gall-bladder
11,461	52	F	0.024	0.023	0.028	0.029	0.079	Chronic ulcerative colitis
11,463	75	M	0.034	0.022	0.015	0.021	0.128	Congenital heart disease
11,464	40	M	0.021	0.029	0.031	0.032		Carcinoma of breast
11,470	53	F	0.028	0.018	0.043			Acute leukemia
11,471	77	F						Carcinoma of gall-bladder
11,472	13	F						
11,475	74	F						

11,476	33	M	0.017	0.010	0.014	0.007	0.048	Hodgkin's disease
11,477	38	F	0.024	0.019	0.011	0.017	0.071	Encephalomyelitis
11,479	28	M	0.021	0.037		0.015		Meningoencephalitis
11,480	64	M	0.046	0.037	0.031	0.009	0.123	Multiple myeloma
11,481	58	M	0.019	0.009	0.015	0.027	0.070	Bacterial endocarditis
11,482	40	F	0.026	0.051	0.026			Carcinoma of breast
11,487	53	M	0.029	0.044	0.023	0.035	0.131	Thrombosis of femoral veins
11,489	70	M	0.019		0.020	0.013		Carcinoma of esophagus
11,491	62	M	0.013	0.016	0.010	0.031	0.070	Rheumatic endocarditis
11,492	67	F	0.023	0.023	0.024	0.042	0.112	Sarcoma of antrum
11,494	62	M	0.030	0.035	0.031	0.022	0.118	Arteriosclerosis
11,495	64	M	0.030	0.065	0.007	0.037	0.139	Duodenal ulcer
11,500	71	M	0.015	0.014	0.008	0.022	0.059	Generalized arteriosclerosis
11,502	67	M	0.029	0.044	0.051	0.067	0.191	Duodenal ulcer
11,505	51	M	0.089		0.033	0.046	0.168	Rheumatic heart disease
11,511	34	F	0.011		0.015	0.027		Pneumonia
11,514	25	M	0.032	0.017	0.020	0.028	0.097	Abscess of brain
11,517	14	M	0.025	0.021	0.028	0.023	0.097	Generalized miliary tuberculosis
11,518	32	M	0.023	0.025	0.025	0.038	0.111	Carcinoma of sigmoid colon
11,519	50	M	0.028	0.040	0.033	0.049	0.150	Carcinoma of sigmoid colon
11,520	23	M			0.025	0.025		Lymphatic leukemia
11,523	52	M	0.022	0.026	0.014	0.040	0.102	Carcinoma of rectum
11,526	58	M	0.009		0.018	0.028		Lobular pneumonia
11,528	81	M	0.017	0.023		0.032		Carcinoma of prostate
11,529	38	F		0.035	0.017	0.055		Acute pancreatitis
11,530	42	F	0.072	0.038	0.112	0.056	0.298	Tetanus
11,533	33	F	0.038	0.040	0.048	0.020	0.146	Tuberculous meningitis
11,535	44	F	0.047	0.046	0.026	0.042	0.161	Hemolytic streptococcus sepsis
11,536	36	F	0.030	0.014	0.029	0.028	0.101	Rheumatic carditis
11,538	48	F	0.017	0.028	0.018	0.022	0.085	Lymphosarcoma of stomach
11,539	82	M	0.041			0.045		Senile arteriosclerosis
11,544	46	M	0.021	0.021	0.021	0.030		Hodgkin's disease
11,545	25	F	0.035	0.032	0.021	0.049	0.137	Cystadenoma of ovary
11,546	60	F	0.040		0.043			Carcinoma of ampulla of Vater, acute pancreatitis
11,547	56	F	0.009	0.025	0.015	0.032	0.081	Acute streptococcus sepsis following angina
11,548	33	F	0.036	0.020	0.022	0.053	0.131	Lymphogranulomatosis inguinalis (rectal)
11,557	57	M	0.014		0.011	0.021		Carcinoma of stomach
11,559	49	M	0.018	0.028	0.015	0.024	0.085	Lead poisoning, thrombosis
11,561	48	M	0.015	0.023	0.020	0.015	0.073	Rheumatic endocarditis, ulcers of duodenum

The calculated total parathyroid weight for males is 0.106 gm. and for females 0.130 gm. The glands from females are thus approximately 20 per cent heavier. If it had been possible to take into consideration the relation to total body weight the difference might have been still greater.

The explanation of this sex difference is not immediately apparent. It does not appear to be correlated with repeated pregnancies. The

TABLE III

Mean Weights of Parathyroid Glands in Non-Nephritic Cases

Gland	No. of cases	Mean weight	Standard deviation
Right upper	62	<i>gm.</i> 0.027 ± 0.0013	<i>gm.</i> 0.013
Right lower	51	0.032 ± 0.0013	0.014
Left upper	59	0.027 ± 0.0013	0.015
Left lower	58	0.031 ± 0.0010	0.012
Combined weight (calculated)			0.117
Combined weight (observed, 37 cases)			0.118

mean weights of the glands in nulliparas are if anything slightly in excess of those in women who have borne children.

On the other hand, if the weights of the glands in women before and after the menopause (arbitrarily taken as 45 years) be calculated separately, the increased mean weight is found to lie entirely in the younger group. This is shown in Table V.

Although the differences in the two groups are not sufficiently great to be statistically significant, except in the case of the left lower, they are consistent for each gland and probably represent a true difference. A similar analysis of the weights of the male glands discloses no comparable difference in the two age groups. The inference which is suggested, if not proved by our data, is that the age period of sexual activity in females is marked by a definite increase in the weight of the parathyroid.

TABLE IV
Influence of Sex on Weight of Normal Parathyroids

	Right upper		Right lower		Left upper		Left lower		Combined weight (calculated)
	No. of cases	Mean weight	No. of cases	Mean weight	No. of cases	Mean weight	No. of cases	Mean weight	
		gm.		gm.		gm.		gm.	
Males	36	0.027 \pm 0.0011	30	0.027 \pm 0.0015	33	0.023 \pm 0.0013	35	0.029 \pm 0.0010	0.106
Females	26	0.031 \pm 0.0006	21	0.037 \pm 0.0008	26	0.030 \pm 0.0008	23	0.032 \pm 0.0012	0.130
Per cent increase females over males		15		37		30		10	22

TABLE V
Mean Weights of Parathyroids in Females Under and Over 45 Years of Age

Age	Right upper		Right lower		Left upper		Left lower		Combined weight (calculated)
	No. of cases	Mean weight	No. of cases	Mean weight	No. of cases	Mean weight	No. of cases	Mean weight	
		gm.		gm.		gm.		gm.	
Under 45	15	0.035 \pm 0.0026	14	0.040 \pm 0.0029	16	0.033 \pm 0.0038	15	0.036 \pm 0.0022	0.144
Over 45	11	0.026 \pm 0.0027	7	0.033 \pm 0.0033	10	0.026 \pm 0.0023	8	0.027 \pm 0.0020	0.112
Per cent increase		35		21		27		33	29

RELATION OF PARATHYROID WEIGHT TO AGE

Taking the series as a whole no correlation has been found between the weight of the parathyroids and the age. This is brought out in Table VI.

While the numbers in each group are too few to permit of statistical analysis, it is evident that there is no definite trend either toward an increase or decrease with advancing age. It should be noted that our data include no cases below the age of 10 and only 3 in the 10-19 year old group.

WEIGHTS OF PARATHYROIDS IN NEPHRITIC CASES

This group may be analyzed first from the point of view of the pathological lesions. The parathyroids were obtained from 27 cases in which at autopsy there were found significant lesions in the kidneys. The data are given in Table VII.

A summary showing in tabular form the mean weights together with the PE_M in the renal cases is given in Table VIII.

Thus, in an unselected series of cases with renal lesions there is found an increase in the weights of the parathyroid as compared with those of a control series. This applies to the individual groups as well as to the total combined weights. This difference is slightly less than three times the square root of the sums of the squares of the probable errors of the means in the cases of the right upper, right lower, and left upper, and slightly greater than three times in the cases of the left lower parathyroids. Strictly, the data are statistically significant only in this last group, according to accepted usage. But the probability that the increased weight in the renal cases is not accidental is enhanced by the fact that it is seen in each comparable group.

Since this series includes indiscriminately various types of renal lesions of various degrees of severity, without regard to duration or to clinical evidence of renal insufficiency, it is probable that the mean differences between the two groups are correspondingly reduced.

Assuming that the increased mean weight of the parathyroid is significant, the question arises as to whether it is due to the inclusion of a few glands of abnormal size or to a general tendency to enlargement. In Chart I are shown distribution curves for the weight of the

TABLE VI
Weights of the Parathyroids at Various Ages

Age	Right upper		Right lower		Left upper		Left lower		Combined weight (calculated)
	No. of cases	Mean weight gm.	No. of cases	Mean weight gm.	No. of cases	Mean weight gm.	No. of cases	Mean weight gm.	
yrs.									
10-19	3	0.022	2	0.025	2	0.030	3	0.025	0.102
20-29	6	0.029	4	0.039	4	0.026	7	0.030	0.124
30-39	8	0.027	8	0.024	9	0.023	9	0.031	0.105
40-49	12	0.031	14	0.038	12	0.034	9	0.030	0.133
50-59	15	0.023	11	0.033	15	0.025	14	0.032	0.113
60-69	10	0.031	8	0.033	10	0.028	8	0.032	0.124
Over 70	8	0.026	4	0.022	7	0.021	8	0.030	0.099

TABLE VII
Weights of Parathyroid Glands in Cases with Renal Lesions

Autopsy No.	Age yrs.	Sex	Right upper gm.	Right lower gm.	Left upper gm.	Left lower gm.	Combined weight gm.	Type of renal lesion	Marked renal insufficiency
11,459	15	F	0.037	0.052	0.060	0.082	0.231	Chronic glomerulonephritis	+
11,456	21	M	0.077	0.052	0.041	0.040	0.178	Subacute glomerulonephritis	+
11,486	43	M	0.025	0.052	0.031	0.050		"	+
11,378	44	F	0.022	0.058	0.013	0.033	0.167	Embolc glomerulonephritis	
11,474	27	M	0.041	0.030	0.035	0.035	0.142	"	
11,594	35	M	0.044	0.196	0.033	0.031		Acute glomerulonephritis	
11,516	40	M	0.042	0.047	0.036	0.074	0.433	Advanced pyelonephritis and renal calculi	+
11,399	38	M	0.038	0.042	0.031	0.035		Pyelonephritis and calculus (left)	+
11,485	29	F	0.042		0.031	0.035		Arteriotar nephrosclerosis	
11,390	54	M		0.047	0.036			"	
11,453	69	F		0.047	0.035	0.074		"	
11,396	46	M	0.033	0.016	0.035			"	
11,400	37	F	0.248	0.016				"	
11,424	49	F	0.037		0.046			"	
11,455	58	M				0.039	0.194	"	
11,553	66	M	0.062	0.065	0.029	0.038		"	
11,556	43	F	0.043		0.040	0.027		"	
11,402	60	F	0.025	0.013	0.035	0.020	0.093	"	
11,398	72	F	0.053	0.034	0.010	0.056	0.153	Arteriosclerotic scars	
11,484	36	F	0.014	0.016	0.014	0.023	0.067	"	
11,496	49	M	0.067					"	
11,420	43	F	0.009	0.036	0.020			Slight hydronephrosis	
11,437	47	M		0.046	0.030	0.033		Marked hydronephrosis	
11,434	59	M	0.013	0.031	0.032	0.067	0.0163	Chronic interstitial nephritis	+
11,488	72	F			0.028			Infarcts of kidneys	
11,453	35	F	0.031		0.017	0.045		Healed infarcts	
11,490	54	M	0.031	0.041		0.026		Calculus, nephrectomy (right)	

TABLE VIII
Mean Weights of Parathyroids in Nephritic and Non-Nephritic Cases

Cases	Right upper		Right lower		Left upper		Left lower		Combined weight (calculated)
	No. of cases	Mean weight gm.	No. of cases	Mean weight gm.	No. of cases	Mean weight gm.	No. of cases	Mean weight gm.	
Nephritic	21	0.047 ± 0.0069	15	0.050 ± 0.0074	22	0.033 ± 0.0018	19	0.047 ± 0.0044	0.177
Non-nephritic	62	0.027 ± 0.0011	51	0.032 ± 0.0013	59	0.027 ± 0.0013	58	0.031 ± 0.0011	0.117
Per cent increase in nephritics		74		56		22		47	50

TABLE IX
Mean Weights of Parathyroids in Cases with Severe Renal Insufficiency

Cases	Right upper		Right lower		Left upper		Left lower		Combined weight (calculated)
	No. of cases	Mean weight gm.	No. of cases	Mean weight gm.	No. of cases	Mean weight gm.	No. of cases	Mean weight gm.	
Severe nephritis	7	0.072	5	0.072	8	0.042	7	0.059	0.244
Controls	62	0.027	51	0.032	59	0.027	58	0.031	0.117
Per cent increase		166		125		56		90	109

glands in the series of nephritic and non-nephritic cases. Because of the relatively small number of nephritic cases the curves are irregular, but it is clearly seen that the greater percentage of the nephritic cases falls to the right of the mode of the controls. Of the right upper glands, 71 per cent exceed the mean weight of the controls; of the right lower, 73 per cent; of the left upper, 73 per cent; of the left lower 74 per cent. In 10 cases in which all four glands were recovered, the total weight exceeded the mean total weight of the controls (118 mg.) in 8. The 2 cases in which the total weight was lower showed only arteriosclerotic scars without clinical evidence of renal insufficiency. Of the 8 cases which were above the normal mean 4 had all glands above the mean and 4 showed enlargement of three glands, indicating that the overgrowth is not limited to one or two of the glands in a given case.

The conclusion which seems justified from this analysis is that the parathyroid enlargement is the expression of a general trend and that the increase in mean weight in the nephritic series is not due to the inclusion of a few glands of exceptional size.

In view of the fact that the female glands average heavier than the males, it should be pointed out that the nephritic series includes 14 males and 13 females. The mean of the male parathyroid is larger than that in the females. It is therefore improbable that the normal sex difference is a factor in the enlargement found in the nephritic cases.

Thus far the discussion has concerned unselected cases of renal disease without regard to the intensity of the lesions or their character. It was of interest to ascertain if there existed a correlation between the degree of parathyroid enlargement and the severity or character of the kidney disease.

Reference to Table VII shows that the maximum enlargement occurred in 2 cases of suppurative nephritis and pyelonephritis in which the combined weight of the parathyroids was approximately four times the normal. The next degree of enlargement was in the 3 cases of subacute and chronic glomerulonephritis, in 2 of which the combined weight of the four glands was approximately double the normal. Lesser increases in weight were found in the nephrosclerotic and other types. In those cases in which the renal lesions were unilateral, acute or focal in character, no enlargement of the parathyroids was found.

An attempt has been made to correlate the degree of enlargement with the severity of the clinical symptoms. From the group of 27 cases showing pathological changes in the kidneys we have selected 9 in which the clinical record gave evidence of severe renal insufficiency.

Comprised in this group are 4 cases of chronic and subacute glomerulonephritis (11,459, 11,456, 11,486), 1 of which (11,399)

PERCENTAGE DISTRIBUTION OF WEIGHTS OF PARATHYROIDS
IN NON-NEPHRITIC AND NEPHRITIC CASES

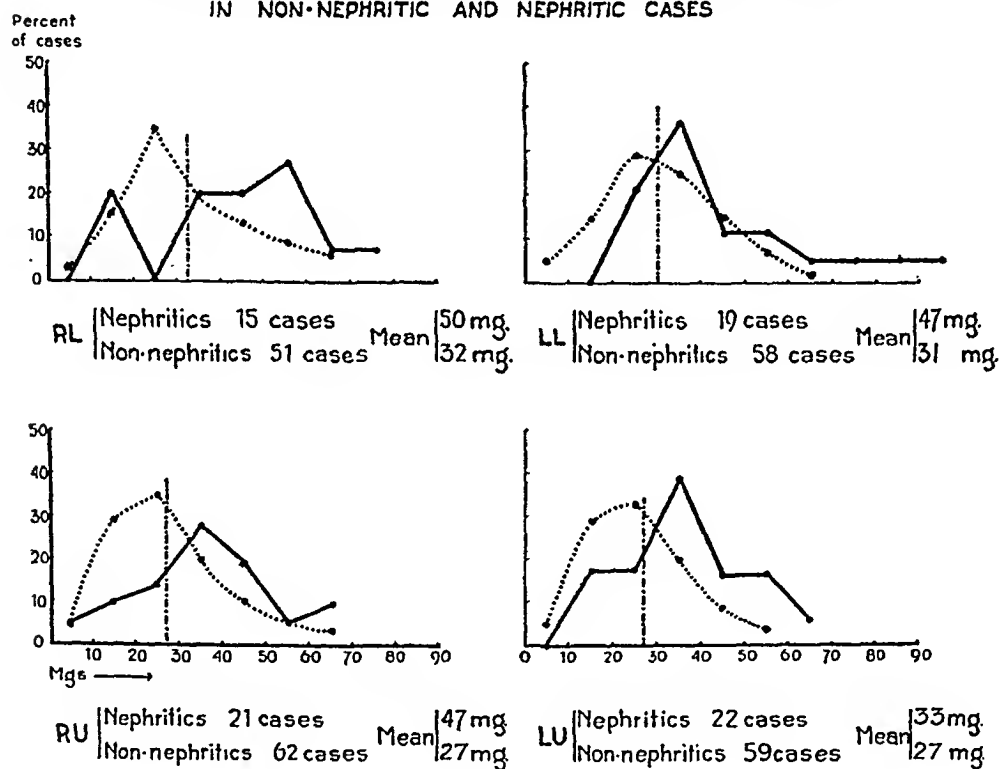


CHART I

also had a renal calculus and pyonephrosis (all died with uremic symptoms); 3 cases of arteriolar nephrosclerosis with hypertension and terminal uremia (11,396, 11,400, 11,556); 1 marked hydronephrosis (11,437) following carcinoma of the bladder with uremia; and 1 case of acute glomerulonephritis (11,516). In spite of the smallness of the series there is rather convincing evidence that the enlargement of the parathyroids is correlated with the severity of the clinical picture. The number of cases in each group is too small and the variations too wide to justify statistical analysis.

DATA ON FIXED SPECIMENS

As confirmatory evidence for the frequent association between parathyroid enlargement and renal disease we may supplement the above findings with data based on the weights of glands obtained from museum specimens. They can be presented most briefly in tabular form (Table X).

Although the actual weights of the glands are reduced by the fixation to about 70 per cent of the unfixed organs, a relative increase in weight is again found in the nephritic series, as compared with the controls. This increase is statistically significant for each group of glands in the severe cases and for the right lower and left upper in the series without clinical evidence of severe nephritis. Since these data merely confirm the observations on unfixed material, it is unnecessary to consider the cases individually.

HISTOLOGICAL CHANGES

The material at hand does not lend itself to detailed cytological study. The primary purpose in sectioning glands was to make sure that no lymphatic or tissue other than parathyroid was included in the weighing. Only a few tentative statements can therefore be made in comparing the histology in the nephritic and non-nephritic cases. In spite of great variability, the impression is obtained that the glands of nephritic cases show a more compact structure and relatively less interstitial adipose tissue than those of the control series. Furthermore, the dominant cell type in most of the "nephritic" glands is the large water-clear cell in which the juxtanuclear body appears conspicuously. Oxyphile cells are not more numerous than in the control glands and indeed seem unusually sparse in some of the nephritic cases.

Definite adenomas were found three times in the nephritic series and twice in the control. They were all of the oxyphile cell type. Further study is needed to determine whether the enlargement of the glands in nephritis is due to hypertrophy of individual cells or to increase in their number.

There have been few systematic studies of the histology of the parathyroid in nephritics. Koopmann¹⁰ examined 5 cases of chronic renal disease, including malignant nephrosclerosis, chronic glomerulonephritis, and a case of cystic disease. No histological

TABLE X
Mean Weights of Parathyroids in Nephritic and Control Cases, Based on Fixed Museum Specimens

Classification	Right upper		Right lower		Left upper		Left lower		Combined weight (calculated)
	No. of cases	Mean weight	No. of cases	Mean weight	No. of cases	Mean weight	No. of cases	Mean weight	
Severe nephritis with renal insufficiency	12	gm. 0.039 \pm 0.0044 77	8	gm. 0.052 \pm 0.0070 108	11	gm. 0.031 \pm 0.0024 72	9	gm. 0.042 \pm 0.0036 68	gm. 0.164 82
Per cent increase over normal									
Renal lesions without clinical nephritis	17	0.027 \pm 0.0029 23	13	0.038 \pm 0.0035 52	14	0.032 \pm 0.0018 78	8	0.037 \pm 0.0038 48	0.134 49
Per cent increase over normal									
Miscellaneous controls	12	0.022 \pm 0.0026	10	0.025 \pm 0.0026	9	0.018 \pm 0.0008	10	0.025 \pm 0.0018	0.090

changes were found which could be correlated with the renal lesions or interpreted as indicating hyper- or hypofunction. No weights or measurements are given.

Radnai ¹¹ also has studied the parathyroids in 20 nephritic cases and 20 controls of similar age groups. He believes that there is a somewhat earlier appearance of oxyphile cells and in greater number in the nephritic cases, but finds no other characteristic change. No weights or measurements are given.

DISCUSSION

The principal facts which have emerged from this study are: (1) the mean weight of the female parathyroid glands is greater than that of males; (2) the mean weight of the parathyroids is significantly increased in any type of nephritis, if the lesions are diffuse and severe. We shall discuss briefly the possible implications of these findings.

The sex difference in the weights of the parathyroids has, so far as we are aware, not been previously observed. The first explanation that comes to mind is that the loss of calcium due to pregnancy or lactation might increase the functional demands upon the parathyroids and lead to their enlargement. Our data do not support this theory, since the enlargement was even more marked in nulliparas than in women who had borne children.

Another possibility is that the increased parathyroid weight may be in some as yet obscure way correlated with the alterations of the anterior lobe of the hypophysis which accompanies the menstrual cycle (Andersen¹²). It has been shown recently by Anselmino, Hoffmann and Herold ¹³ that injection of anterior pituitary lobe extract in rats is followed by a rapid hypertrophy of the parathyroids, with a characteristic change in the cytological picture. Hertz and Kranes ¹⁴ have reported similar effects in rabbits. Whether this is a reversible change or not is not known; nor has it been demonstrated yet that this activity of the anterior lobe is due to a special hormone. It is obvious that the exact explanation for the sex difference in parathyroid weights in humans must await further experimental study.

Our observations have brought out clearly the fact that most, if not all, cases of diffuse renal disease are accompanied by a significant

enlargement of the parathyroid glands. Since this occurs in such varied types of renal disorders as glomerulonephritis, arteriolar nephrosclerosis, hydronephrotic atrophy and suppurative pyelonephritis there must be some common chemical factor that stimulates the parathyroids to increased activity and growth.

It seems hardly justified to enter upon an extended discussion of the nature of this correlation on the basis of the data here presented. It is probable that the cases with severe clinical nephritis had phosphate retention, and since "any increase in PO_4 ions will decrease the amount of Ca ions in the blood" (Thomson and Collip¹⁵), this may incite the parathyroids to increased activity and overgrowth. Whether this simple explanation is adequate or not must be determined by further clinical and experimental studies; our data include only a few determinations of inorganic PO_4 and Ca in the nephritic series, and no conclusions as to a positive correlation can be drawn from them.

It is interesting that the group of cases in which renal lesions were found at autopsy, but in which symptoms of renal insufficiency were not recognized, nevertheless showed in most cases a certain degree of parathyroid enlargement.

It is unfortunate that the bones in this series of nephritic cases could not be carefully studied. Although in none of the cases was there any clinical or gross pathological suspicion of bone disorder, it is possible that those cases in which the parathyroid enlargement was most pronounced might have shown microscopic lesions indicating increased resorption. In renal dwarfism, in which the kidney disease leads often to extreme rickets-like deformity of the skeleton, the parathyroids have not been carefully studied. Langmead and Orr,¹⁶ however, have reported a case in which there did occur parathyroid enlargement and they suggest that the bone changes may have been due in part to excessive parathyroid activity. It may indeed be true that excessive activity of the parathyroids during the growth period may bring about more severe skeletal deformities than in adult life.

In the series of cases which we have analyzed it seems obvious enough that the diverse renal lesions could not have resulted from excessive functional activity of the parathyroids. In the group of cases of hyperparathyroidism collected by Albright, Baird, Cope and Bloomberg² it is taken for granted that the renal lesions found in

over half the cases are attributable to the excessive activity of the parathyroids, and in large measure are due to calcium deposition. They hold that the precipitation of calcium phosphate in the renal parenchyma eventually leads to inflammatory changes, sclerosis and contraction, which simulate both chronic glomerular and vascular nephritis. We believe that few pathologists would accept this without question. In some of the cases cited the deposition of calcium in the renal tissues may well have been due to the hyperparathyroidism, and may have been entirely unrelated to a preëxisting nephritis. It is possible, then, that a certain proportion of cases of so-called hyperparathyroidism may be initiated by chronic renal disease.

SUMMARY

1. The mean weights of the parathyroid glands in a series of miscellaneous non-nephritic cases over the age of 10 years is 27 mg. for the upper parathyroid and 31-32 mg. for the lower. The mean combined weight is 118 mg.

2. In the male glands there is no change correlated with advancing age.

3. In the female gland there was found an increase in weight of approximately 22 per cent during the active sexual period; after 45 years there is a decline of weight to figures corresponding with those of the series as a whole. The enlargement is not correlated with pregnancy.

4. The mean weight of the parathyroids in various types of chronic renal disease exceeds that of non-nephritic cases. In an unselected series this increase in mean weight is approximately 50 per cent; in cases with advanced renal lesions the increase amounts to more than 100 per cent.

5. The increase in weight of parathyroids is roughly proportional to the severity and extent of the renal lesions and to the intensity of the clinical signs of renal insufficiency. Usually three or four of the glands share in the enlargement.

NOTE: We desire to express our thanks to Dr. Walter W. Palmer and to Dr. Allen O. Whipple for permission to utilize the clinical records in these cases.

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ATYPICAL AMYLOID DISEASE *

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The following cases of extensive amyloid disease are reported because they possess several unusual features. Suppuration, tuberculosis and malignancy with infection were absent in all. Primary amyloid disease, so-called because of the absence of any known etiological factor, is a rare condition and only a few cases have been reported in the literature.

Of 1500 autopsies performed at the Montefiore Hospital during the past 7 years 112 showed evidence of amyloid disease; 100 of these were associated with pulmonary tuberculosis. This represented 25 per cent of all patients dying with this disease. Of the 12 cases of amyloidosis in patients dying with diseases other than pulmonary tuberculosis 3 had no apparent etiological factor, 2 were associated with carcinoma of the lung and secondary suppuration, 1 was associated with chronic osteomyelitis, 3 with pyelonephritis, 1 with carcinoma of the stomach, 1 with leukemia, and 1 with tabes dorsalis. The more unusual of these cases are reported in this communication.

Lubarsch,¹ in a discussion of atypical amyloid deposition, noted certain characteristics of this group of cases. (1) There is almost complete absence of amyloid in those organs that are most involved in ordinary amyloidosis (liver and spleen). (2) Organs such as the heart and lungs, the skin and striated muscle, not affected generally in amyloidosis, are particularly involved. (3) The amyloid may occur in the form of discrete nodules (Eppinger's case). (4) Frequently the deposits fail to react to the well known tests for amyloid. (5) There is no demonstrable concomitant infection such as is found in the typical amyloidosis.

Lubarsch reported 3 cases of atypical amyloidosis. (1) A case of chronic endocarditis of the mitral valve in which amyloid deposits were found in the heart, lung, stomach, esophagus, small and large intestine and skin. In this case there was, however, a mild

* Received for publication June 20, 1934.

ascending genito-urinary infection. (2) A male 53 years of age with symptoms and signs of scleroderma, myotonia and marked macroglossia, which was diagnosed as carcinoma of the tongue. This proved to be due to massive amyloidosis of the tongue. (3) A male 45 years of age with multiple ulcerations of the stomach, hemorrhagic cystitis with marked amyloidosis of the spleen, trachea, bronchial and mesenteric lymph nodes, prostate, seminal vesicles, epididymis, testes, stomach, heart muscle and lungs. The only suppuration or infection was an antecedent gonorrhea which had completely disappeared.

Recently Gerstel² reported a case of diffuse amyloidosis in a female 52 years of age who, after the use of a denture, observed swelling of the floor of the mouth. The tongue became so large that she could not close her mouth. A sarcoma of the tongue was suspected. The entire tongue was hard and swelling extended to the neck. The patient became progressively weaker and during the 3 months preceding death developed increasing dyspnea, edema of the lower extremities and severe diarrhea. Examination revealed an emaciated woman with generalized anasarca. Her lids were swollen and yellowish brown. At autopsy diffuse amyloidosis was found involving the tongue, skin of the neck, esophagus, pylorus, intestine, heart and adventitia of large vessels. There was no evidence of inflammation in any of the organs and no definite cause could be found to account for the amyloidosis. The striking features of the case were the rapidity of the development of the amyloid of the tongue, resulting in death in 2 years, and the unusual dysentery-like symptoms due to amyloid of the gut.

Pick,³ in discussing amyloid disease, mentioned a case of a man of 54 years who, 8 years prior to his death, developed symptoms of difficulty in digestion and deglutition and progressive difficulty in moving the tongue. At autopsy widespread amyloid of the musculature was found, particularly of the tongue, esophagus, heart, stomach and intestine. Nowhere was the mucous membrane involved. The lung and serous membranes were likewise unaffected. This condition gave the picture of pseudoscleroderma and macroglossia.

Three unusual cases of amyloid disease were recently reported from the Mayo Clinic by Bannick, Berkman and Beaver.⁴ One case was associated with lymphosarcoma, another with gastric carcinoma

and in a third there was apparently no etiological factor of suppuration.

On the basis of the cases reported in the literature Lubarsch classified the atypical cases of amyloid into (1) amyloid with pseudo-scleroderma, (2) amyloid with pseudomyotonia, and (3) amyloid with massive involvement of tongue, simulating neoplasm. There is no particular advantage in such an attempted classification.

In reporting a case of diffuse amyloidosis with deposition in unusual sites, Strauss⁵ suggests the term "paramyloidosis" for this group of cases. He believes that in this type the amyloid is more frequently deposited in the musculature of the arterioles and small arteries of various organs and in mesenchymal tissue instead of in pericapillary and periglandular sites, as in typical amyloid disease. He collected 27 cases from the literature illustrative of unusual deposition of amyloid.

CASE REPORTS

CASE 1. B. H., a female, aged 53 years, was admitted with a history of dyspnea, cough and pain in the chest of several months duration, associated with loss in weight and increasing weakness. Six months prior to admission she had an attack of bronchopneumonia and an acute serofibrinous pleurisy. On admission the patient showed cyanosis of the lips and there was evidence of ascites. The heart sounds were distant and of poor quality, the rhythm regular and a systolic murmur was heard at the apex. The red blood cell count was 1,990,000, hemoglobin 40 per cent, white blood cells 10,400. The blood pressure was 104/80. The blood Wassermann test and the other laboratory findings were negative.

Two weeks after admission the patient developed a sudden attack of dyspnea, became cyanotic, her breathing became stertorous and she went into collapse and died on June 15, 1932.

The clinical diagnoses were congestive heart failure and (?) carcinoma or malignant tumor of the lung.

Postmortem Examination

The anatomical diagnoses were primary amyloid disease involving chiefly the heart, tongue, kidneys, lungs and gastro-intestinal tract; hypertrophy and dilatation of the heart; mural thrombi in right and left auricles; edema of the lower extremities; ascites; hydrothorax, bilateral; congestion, edema and partial atelectasis of the lungs; recent thrombosis of renal veins; arteriosclerosis of the kidneys.

The body was that of a well developed, poorly nourished, elderly white female in partial rigor. There was slight edema of the lower

extremities. A few hundred cc. of straw-colored fluid were present in the abdominal cavity. A little over a liter of clear straw-colored fluid was present in each pleural cavity. The lungs were partially collapsed. There were no adhesions.

Heart: The heart weighed 390 gm. The measurements were: pulmonic ring 7 cm., aortic ring 6.7 cm., mitral ring 9.2 cm., tricuspid ring 10.5 cm., left ventricular wall 12-13 mm., right ventricular wall 3-5 mm. The pericardium was normal. The heart had a peculiarly firm consistence. The left border was markedly rounded. On section the myocardium offered considerable resistance to cutting and presented a pale, grayish, waxy surface marked with translucent grayish streaks and with opaque yellow spots and streaks. A strongly positive test for amyloid was obtained with Lugol's solution.

In the left auricular appendage there was a soft mural thrombus with a smooth grayish surface and a soft reddish center. A few yellow, atheromatous patches were present in the aortic leaflet of the mitral valve. The left and right ventricles were considerably hypertrophied and slightly dilated. A few yellow, atheromatous patches were present in the sinuses of Valsalva. The valves were otherwise normal. Adherent to the pectinate muscles were three globular thrombi with a smooth gray surface and a soft reddish center, which ranged in size from 8 to 18 mm. The coronary arteries were smooth and widely patent.

Lungs: The lower lobe of the right lung was collapsed, non-crepitant, firm in consistence, dull reddish gray in color, and yielded a small quantity of clear fluid on pressure.

The left lung showed extensive atelectasis involving the entire lower lobe and the lower quarter of the upper lobe. Lugol's solution applied to the lung tissue apparently yielded a positive test for amyloid. The hilum and tracheobronchial lymph nodes were large and anthracotic.

Liver: The liver weighed 1050 gm. and measured 24 by 15.5 by 7 cm. The capsule was smooth. On section the surface was pale brownish and moderately congested. The test with Lugol's solution for amyloid was negative.

Spleen: The spleen weighed 110 gm. and measured 12.5 by 7 by 4 cm. The capsule was smooth, the organ firm, and on section the surface was purplish red. The markings were distinct. Amyloid test was faintly positive.

Kidneys: The right kidney weighed 105 gm. and measured 9.5 by 4.5 by 3 cm. The left weighed 110 gm. and measured 10.5 by 4.5 by 3 cm. The cortex measured 3-4 mm. and the medulla 15-18 mm. The kidneys were firm and decreased in size. The capsule stripped with ease, leaving a strikingly mottled, pale reddish yellow, coarsely granular surface with a number of larger shallow scars. On section the cortex was narrowed and uneven, pale yellowish and somewhat waxy in appearance. The markings were distorted. The cortex and the medulla were sharply differentiated. The test for amyloid was strongly positive. The renal veins, beginning about 1 to 2 cm. beyond the hilum of each kidney, were completely occluded by soft, friable, red and gray, partly lamellated thrombi, which involved also the ramifications in the kidney sinuses (Figs. 1 and 2).

Suprarenals: The right weighed 8.5 gm. and the left 9 gm. The cortex was bright yellow and the medulla somewhat autolyzed. The iodine test for amyloid was faintly positive.

A positive amyloid test was obtained in the bladder wall, vaginal wall, uterine wall and ovaries.

Lugol's solution yielded a dark brown color in the muscularis of the esophagus, stomach and intestines and dark brown spots in the mucosa of these organs.

Neck Organs: The tongue was definitely enlarged, due chiefly to a pronounced increase in thickness, and was unusually firm in consistence. On section it presented a strikingly pale yellowish brown surface with translucent grayish streaks. A strongly positive amyloid reaction was obtained. The mucosa of the epiglottis, larynx, trachea and main bronchi was pale. Lugol's solution applied to these structures yielded brownish spots (Fig. 3).

Bone: Sections of vertebrae and ribs presented no gross abnormalities.

Skeletal Muscle: Sections of diaphragm, psoas muscle and muscle of anterior abdominal wall gave a positive reaction for amyloid with Lugol's solution.

Microscopic Examination

Heart: Section of the *right auricle* shows most of the muscle replaced by a homogeneous pink-staining material. Among the trabeculations of the endocardium are thrombotic masses.

Section through *left ventricle* shows a diffuse infiltration of the myocardium with a peculiar homogeneous pink-staining material (amyloid). In these areas the musculature is atrophic. Muscle cells of the surrounding portions appear to be hypertrophic. In the areas of amyloid accumulation, muscle cells have undergone pressure necrosis. There is no cellular reaction, however, to the necrosis. In other sections of the heart amyloid material is present beneath both the endocardium and the pericardium (Fig. 4).

Lung: Marked atelectasis of the lung is seen. Many of the blood vessels contain peculiar homogeneous material in their walls which varies in amount in different parts of the lungs, but is particularly prominent in the small vessels. Amyloid material is present in the alveolar walls (confirmed by Congo red test).

Liver: Congestion of the central portions is present with vacuole degeneration of the nuclei of the cells. Some increase of the periportal connective tissue is seen. The walls of the arteries in the portal zones are thickened and strikingly infiltrated with amyloid (Congo red test). No amyloid is present in the parenchyma.

Spleen: The follicles are prominent and in many there is a peculiar homogeneous pink-staining material (amyloid). The vessels are somewhat thickened and amyloid is deposited in the walls of the arterioles (confirmed by Congo red test). The sinuses are markedly congested.

Tongue: There is extensive amyloid replacement of the muscle tissue. Striated muscle is everywhere atrophic and compressed by stringy, pink-staining, acellular material (Fig. 5).

Kidney: Extensive thickening of the capsule is present with numerous areas of scar tissue formation with extensive atrophy of tubular elements and glomeruli and round cell infiltration. Connective tissue about the scars has a pink-staining, homogeneous appearance. A few of the glomeruli are replaced by connective tissue arranged concentrically and some of these areas are hyalinized. Many of the tubules are distended with casts of albuminous material. Intervening blood vessels are congested and arterioles are everywhere markedly thickened and the lumens narrowed. Congo red test for amyloid is positive.

Colon and Stomach: Both show some replacement of the smooth muscle with acellular pink-staining material. Amyloid is also present in the submucosa. None is present in the wall of the gall-bladder.

Diaphragm: Shows striking atrophy of striated muscle and varying degrees of degeneration with pink-staining material present in the interstitial tissue.

Uterus: Amyloid is deposited in the walls of medium sized and small vessels and occasionally between the muscle fibers. In all instances the presence of amyloid in the tissues was confirmed by the Congo red test.

Comment

The unusual features of this case are the macroglossia due to amyloid infiltration of the tongue, the marked amyloid disease of the heart, lungs, colon, diaphragm and uterus, and the extensive dilatation and hypertrophy of the heart which eventually resulted in cardiac insufficiency and death. This resembles the case of Gerstel and Pick. In this case no infection of any kind was demonstrable. The cardiac hypertrophy was due probably to previous hypertension associated with renal arteriosclerosis. The presence of extensive amyloid disease in the myocardium is not responsible for the hypertrophy of the heart, in our opinion. Replacement of functioning cardiac musculature would not lead in itself to cardiac hypertrophy. Extensive fibrosis following coronary disease does not cause cardiac hypertrophy. No doubt, however, the extensive replacement of the heart muscle by amyloid contributed to myocardial insufficiency.

CASE 2. G. C., a female, aged 16 years, was admitted to the hospital May 4, 1930, complaining of pain in the joints of 10 years duration, associated with fatigue, general weakness, dyspnea at rest, and palpitation, and during the past 2 years progressive increase in size of the abdomen. Occasionally the joints were swollen and reddened. No fever was noted.

On examination the patient was found to be underdeveloped, poorly nourished and pale. A rachitic rosary with flaring of the costal margins of the ribs was present. The heart was somewhat enlarged and a blowing systolic murmur was heard at the apex. Blood pressure was 124/80. The abdomen was large and the superficial abdominal veins were prominent. The liver edge could be felt 10 cm. below the costal margin, the spleen 4 cm. below the costal margin. There was some limitation of motion of the right elbow and left wrist. The axillary, submaxillary and inguinal lymph nodes were enlarged.

At the time the patient was admitted the red blood cell count was 2,500,000, hemoglobin 47 per cent, white blood cell count 7500. The Van den Bergh reaction was delayed and slightly positive (0.4 mg. per cent). The Congo red test showed a retention of 88 per cent of the dye after 1 hour. The blood urea nitrogen was 69.2 mg. per 100 cc., creatinine 2 mg. per 100 cc. A marked decrease in the urea concentrating power was found. The renal concentration showed a

specific gravity of 1.008-1.010. The urine contained a large amount of albumin and was loaded with hyaline and granular casts. The phenolsulphonephthalein test showed a 10 per cent output of the dye in 2 hours. The blood serum calcium was 8.9 mg. per cent, the blood serum phosphorus 8.3 mg. per cent. Successive determinations showed increases in urea nitrogen and creatinine concentration. A diagnosis of chronic glomerulonephritis with renal insufficiency and uremia was made.

During her stay in the hospital the patient had several attacks of arthritis with involvement of the ankles. Ten months after admission she had sudden epistaxis, vomited blood and became hyperpnoeic. A tachycardia was noted, and purpuric spots appeared on the arms. The fundi showed slight swelling of the discs. The urea nitrogen was 108.6 mg. per 100 cc., the creatinine 7.3 mg. per 100 cc. and the CO₂ combining power 17.6 volumes per cent. The CO₂ combining power several days later was 54 volumes per cent, the urea nitrogen rose to 154.1 mg. per 100 cc. with a creatinine of 10.5 mg. per 100 cc.

The spinal fluid was under slightly increased pressure. Several days later the patient had a convulsion and on examination a bilateral inexhaustible clonus was elicited. During the same period an acute purulent parotiditis developed. Following incision and drainage the parotiditis cleared up and the patient improved. Apathy continued with occasional attacks of fever. From December 1931 until April 1932 three transfusions were given. Purpuric spots were again noted in July 1932. Attacks of paroxysmal dyspnea associated with gallop rhythm and wheezing râles in the chest occurred during August. Late in August, 2 years and 4 months after her first admission, she developed signs of myocardial insufficiency with enlargement of the liver and pulmonary congestion. The blood pressure rose to 180/94. The patient was orthopneic and her condition became progressively worse, pallor increased and gallop rhythm persisted. A precordial friction rub was heard over the sternum and on Aug. 29, 1932, the patient became markedly dyspneic and died.

The clinical diagnoses were chronic glomerulonephritis with progressive renal insufficiency and uremia; myocardial insufficiency, gallop rhythm and congestive heart failure; ankylosis of right elbow, partial in left wrist, and recurrent acute arthritis.

Postmortem Examination

The anatomical diagnoses were amyloidosis of kidneys with contraction; amyloidosis of suprarenals and liver; hypertrophy and dilatation of heart; fibrinous pericarditis; chronic passive congestion of lungs, liver, spleen and gastro-intestinal tract; juvenile rickets (clinical); ankylosis of right elbow joint; infantile genital tract.

The body was that of a poorly developed and poorly nourished white female, 142 cm. in length, in complete relaxation. Pelvic and axillary hair was totally absent. The breasts were small. There was marked pallor of the skin and mucous membranes with a subicteric tint. The face was puffy and the neck veins prominently distended. There were scattered purplish blue blotches on the skin of the an-

terior neck and upper extremities. About 150 cc. of serosanguineous fluid were present in either pleural cavity. There were few adhesions.

Heart: The heart weighed 350 gm. The measurements were: pulmonic ring 6.5 cm., aortic ring 6 cm., mitral ring 9 cm., tricuspid ring 9.5 cm.; right ventricle measured 6 mm., left ventricle 18 mm. The pericardial sac contained no fluid. A thin fibrinous deposit was present over the midportion of the anterior wall of the left ventricle and adjacent right ventricle. There were moderate dilatation and hypertrophy of right and left auricles and right ventricle. There was marked hypertrophy with dilatation of left ventricle. Valves and coronary arteries were normal. The myocardium had a dull, light red color.

Liver: The liver weighed 1320 gm. and measured 24 by 18 by 7 cm. The capsule was smooth and the parenchyma a diffuse dull brown with an icteric tint.

Spleen: The spleen weighed 380 gm. and measured 16 by 11 by 5 cm. The organ was enlarged, firm and rubbery in consistence. The capsule was smooth. On section the parenchyma was deeply congested and the markings indistinct. The pulp did not scrape away. Iodine test for amyloid was negative.

Kidneys: Each kidney weighed 50 gm. and measured 8.5 by 5 by 2.5 cm. The cortex measured 2 to 3 mm. and the medulla 12 to 14 mm. The organs showed an identical and striking picture, being markedly contracted and firm. The capsule stripped with slight difficulty, leaving a light, yellowish red surface dotted with many minute, pin-point, glassy and grayish, slightly raised elevations between which were innumerable pin-point to pin-head-sized yellowish dots and streaks. The organs cut with increased resistance. In some places the cortex was narrowed, measuring 2 to 3 mm., and was not sharply delimited from the medulla. Cortical and medullary architecture was replaced by numerous fine and coarse, irregular, light yellowish streaks. The parenchyma had a waxy appearance and gave a strongly positive iodine test. There were a few petechiae in the pelvic mucosa.

Suprarenals: The right suprarenal weighed 10 gm., the left 9 gm. The organs were enlarged and firm. There were several adenomatous cortical nodules of similar appearance, the largest measuring 0.5 cm. in diameter and about 3 mm. in width. The cortex was dull gray and waxy in appearance. The medulla appeared normal. Amyloid reac-

tion was positive in the cortex. There was no evidence of amyloid in the gastro-intestinal tract, including the tongue.

Elbow Joint: The right elbow joint was ankylosed in a position of 170° extension, due to thickening and shortening of the capsule. Numerous thin, easily torn, fibrous adhesions extended between the borders of the articular surface of the humerus and ulna. The cartilaginous articular surfaces were slightly rough, but there were no adhesions between them.

Microscopic Examination

Heart: Hypertrophy of fibers and nuclei, with marked cloudy swelling and fragmentation is present. There is moderate increase of interstitial tissue with foci of lymphocytic infiltration. Epicardial fat is increased and contains many scattered lymphocytes, plasma cells and monocytes. The intima of the main coronary artery shows slight subendothelial swelling, lipoid infiltration with degeneration and hyalinization.

Liver: Marked parenchymatous degeneration and congestion is seen. There are areas in which the entire lobule, except for a narrow zone at the periphery, is completely destroyed and replaced by pink-staining, fibrillar substance in which there are occasional mononuclear cells with hemosiderin, scattered erythrocytes and ghosts of preëxisting hepatic cells. At the periphery there are a few red blood cells in the parenchyma suggesting recent hemorrhage. There is slight increase of periportal connective tissue with lymphocytic infiltration. The arterioles are thickened.

Spleen: The sinuses are markedly dilated and congested and the pulp is atrophic. Malpighian corpuscles are fairly prominent.

Kidney: The glomeruli are closely crowded, large and replaced, except for a few scattered nuclear elements, by amyloid. The glomerular tufts are fused with the capsule which is also thickened by amyloid. There is a marked diffuse increase of interstitial tissue. The great majority of the tubules are markedly atrophic. However, there are areas in which the tubules are widely and irregularly dilated, evidently compensatory. There are frequent, small, focal collections of lymphocytes and plasma cells in the interstitial tissue. The arterioles show considerable thickening with amyloid and narrowing or obliteration of the lumens. The interlobular and arcuate

arteries show fairly marked medial hypertrophy with subendothelial deposition of a lipoid substance. Other arteries of the same size show complete obliteration of the normal architecture with a fibrillar acellular tissue, probably hyalinized connective tissue (Fig. 6). In some areas there are calcific deposits in the medulla, which seem to have replaced tubular epithelium.

Suprarenal: Sections show almost complete replacement of cortex and medulla by amyloid material. There are occasional small islands in the zona glomerulosa and in the medulla which are spared, but these show extensive degeneration. Within the cortex there are occasional calcific plaques.

Pituitary: The anterior portion contains a few, small, irregular cysts filled with amorphous, pink-staining material and lined by a single flat layer of basophilic or eosinophilic cells. There seems to be a slight increase of basophilic cells.

Comment

This case presents several unusual features. The only infection was chronic arthritis, which was not suppurative, with ankylosis of one joint. At the time the complicating parotiditis occurred, evidence of uremic symptoms were manifest. Amyloidosis in a child of 16 years is in itself rare. Amyloid was deposited in the kidneys, suprarenals and liver. The severity of the amyloid deposition in the kidney led to marked renal insufficiency and hypertension, and the patient died of uremia. Uremia, complicating amyloidosis of the kidney, has been observed not infrequently, but it is beyond the scope of this paper to review this problem.

CASE 3. S. S., female, aged 63 years, admitted April 13, 1933, complaining of progressive asthenia, constipation and anorexia, and loss of 50 pounds in weight during the preceding 2 years. During this period she had frequent attacks of diarrhea with watery and bloody stools accompanied by rectal tenesmus. For some time prior to admission she complained of severe headaches and a year before admission to the hospital had suffered a temporary loss of memory associated with mental confusion and disorientation, which at the time was attributed to a cerebral insult. For 6 months she had complained of epigastric pain. At another institution signs of congestive heart failure, pulmonary edema, right hydrothorax and a gallop rhythm had been found. The electrocardiogram showed a cove plane T₁. The blood urea was 30 mg.

On admission to the hospital there was slight cyanosis of the lips, moderate dyspnea and the carotid arteries were markedly sclerotic and tortuous. The heart was enlarged to the left. A systolic apical murmur was heard at the apex

and gallop rhythm was present; the apical primary sound was reduplicated. The superficial peripheral vessels were sclerotic. The epigastrium was tender and the liver edge was felt three fingers below the costal margin. A midline low abdominal scar was present. The blood pressure was 122/86. The fundi showed narrow shiny vessels, degenerative changes in the macula attributed to anemia.

On fluoroscopy the heart was horizontal, the left ventricle moderately enlarged; the other chambers were thought not to be enlarged. The ascending limb of the aorta was elongated, the supraventricular portion dilated, the entire descending limb being greatly dilated with a circumscribed bulge in its middle third. The aorta gave a picture suggestive of localized aortic aneurysm. Small pleural effusions were found at both bases.

Thoracentesis had to be repeatedly performed, with removal of from 700 to 1000 cc. of fluid from each side.

On June 17, 1933, 2 months after admission the patient complained of substernal oppression and dyspnea. The liver edge was felt two fingers below the costal margin. The fluid reaccumulated so that thoracentesis had to be repeated.

Progressive azotemia appeared and vomiting continued. The urea nitrogen, which was 34.6 mg. per 100 cc. on admission, rose to 101.6 with a creatinine concentration of 10 mg. per 100 cc.; blood proteins were reduced, the serum albumin, being 2.76 mg. per cent, globulin 1.90 mg. per cent, the blood serum calcium 7.9 mg. per cent, phosphorus 8.7 mg. per cent, and the blood CO_2 35 cc. per 100 cc.

Generalized anasarca became more marked and on July 27, 1933, the patient developed a marked tachycardia, which by electrocardiogram proved to be auricular flutter with a 2:1 block. The same day an impure flutter with fibrillation appeared. On 22 cat units of digitalis regular rhythm was restored within 48 hours.

Despite treatment edema persisted and simultaneously the total blood proteins were further reduced to a serum albumin of 2.53 mg. per cent and a globulin of 1.46 mg. per cent. The blood urea rose to 109.2 and the creatine to 11.3 mg. per cent.

On Sept. 4, 1933, she had a generalized convulsion and following the convulsive seizure was comatose, had a tachycardia, and the blood pressure fell to 82/55. A few minutes later, however, she came out of the coma, was incontinent of urine, became very weak, vomited and died suddenly on Sept. 5, 1933.

The clinical diagnoses were (?) carcinoma of the gastro-intestinal tract, coronary artery disease and vascular renal disease with uremia.

Postmortem Examination

The anatomical diagnoses were amyloid contracted kidneys; thrombosis of renal veins (bilateral); generalized arteriosclerosis; aneurysm of descending aorta (arteriosclerotic); atherosclerosis of coronary arteries with occlusion of left anterior descending branch; healed myocardial infarction of left ventricle and interventricular septum; mural thrombus of left ventricle; chronic passive congestion of liver and spleen; adenomas of thyroid; ulcers of duodenum, and bilateral hydrothorax.

The body was that of a poorly nourished and poorly developed,

elderly white female about 155 cm. in length, moderately emaciated and in semirigor with moderate edema of arms, lower extremities and back, and with slight cyanosis of lips and finger tips. There were some moderately firm adhesions of omentum to the parietal peritoneum of the anterior abdominal wall. About 100 cc. of clear yellow fluid were present in the pelvis. The uterus was retroverted, and the tubes were matted in a mass of adhesions to the rectal wall of the cul de sac. The diaphragm was at the level of the fourth space on the right and at the fifth space on the left. Each pleural cavity contained about 1500 cc. of clear, amber-colored serous fluid.

Heart: The measurements were: pulmonic ring 7.5 cm., aortic ring 7 cm., mitral ring 8.5 cm., tricuspid ring 11 cm., left ventricular wall 17 mm., right ventricular wall 1 to 2 mm. There were no adhesions. The right border was made up entirely of right auricle. There was a marked increase in amount of epicardial fat over the right side of the heart. The organ was soft and flabby. The apical region of the left ventricle and lateral surface adjacent to it sank in so that a depressed area 3 cm. in diameter was visible on this lateral aspect of the left ventricle. The myocardium was grayish red with many fine gray streaks throughout. There was marked thinning of the myocardium in the lower half of the interventricular septum and in the lateral aspect of the left ventricle and the apical region corresponding to the depressed area on the surface. Adherent to the lower half of the interventricular septum in the left ventricle was a firm, grayish pink, oval mass about 3.5 by 2.5 cm. protruding slightly into the ventricular cavity. It cut with ease and in the depths adjacent to the thinned-out muscle it was broken-down and yellow. At the junction between the adherent clot and the thinned myocardium was a fine layer of firm, yellow, apparently calcified material. The endocardium of the entire left ventricle was markedly thickened, white and opaque. Gray streaks were visible through the endocardium of the right ventricle over the region of the interventricular septum. There was no hypertrophy or dilatation of any of the chambers. The valve leaflets of the tricuspid, aortic and pulmonic valves were normal. The aortic leaflet of the mitral valve was thickened by a raised, yellow, atherosclerotic plaque. There was slight thickening at the base of the mitral valve leaflets.

The coronary arteries were thickened and tortuous. There were raised, calcific, atherosclerotic plaques in their walls. The anterior

descending branch of the left coronary was markedly narrowed in its proximal third and in one area completely occluded by a yellowish calcific mass which contained a fine recanalized lumen.

Lungs: Both were rather small, having been compressed by fluid. They were crepitant throughout, mottled grayish black and cut with ease. The cut surface was reddish gray. In several small branches of the pulmonary artery there were adherent, grayish yellow, firm clots.

Liver: The liver weighed 800 gm. and measured 19 by 17 by 7 cm. The organ appeared smaller than normal, was firm and of a light brown color. The capsule was smooth and cut with normal resistance, revealing well defined hepatic markings with alternating brown and yellow streaking. Amyloid test with iodine was negative.

Spleen: The spleen weighed 100 gm. and measured 9 by 7 by 3 cm. The organ was small, firm and rubbery and cut with increased resistance, revealing a reddish purple cut surface with distinct markings. The pulp did not scrape away. The amyloid test was negative.

Kidneys: Each weighed 100 gm. and measured 8 by 3.5 by 2 cm. The cortex measured 3 mm., the medulla 11 mm. The organs were small and firm, and the capsule stripped easily, revealing a finely granular, grayish pink and yellow surface. They cut with resistance, the cut surface showing a ground-glass, yellowish pink appearance. The markings were poorly defined, the cortex was narrow and poorly differentiated from the medulla. The small vessels gaped slightly. Arcuate veins were occluded by grayish pink, firm clots. There was a strongly positive amyloid test with iodine. The pelves and ureters were normal. Both renal veins were occluded by grayish pink, moderately firm material. Occluding tissue extended into the veins of the parenchyma but did not extend out to the junction of the renal vein with vena cava.

Pelvic Organs: The tubes were adherent to the posterior wall of the cul de sac. The ovaries could not be definitely located but they may have been incorporated in the adhesions. Uterus and bladder were normal.

Thyroid: Of normal size and shape, weighing 22 mg. At the lower pole of the right lobe was a hard mass 2.5 cm. in diameter, apparently encapsulated, which on cut section was yellow and quite firm. The thyroid tissue was pink and contained numerous small, grayish pink nodules, which were softer than the surrounding parenchyma. A

few of these contained grayish fluid. Others were solid, and a few were stony hard.

Blood Vessels: The entire aorta was inelastic and was the seat of marked atherosclerotic changes. The ascending portion was dilated from above its origin to the arch, forming a slight pouch. In this region the aorta was elastic and contained relatively few raised yellow flecks. The arch and openings of the cephalobrachial vessels showed more numerous raised areas, a number of which were firm and hard. The descending aorta from the arch into the vessels of the lower extremities was markedly thickened and there was very little normal intima visible. The intima was replaced by yellowish hard plaques and shallow, irregular ulcerated areas covered with clot and irregular, hard, shell-like flattened tissue. At the region of bifurcation calcification and ulceration were most marked. In the midportion of the descending thoracic aorta was a knob-like protrusion to the left, about 4.5 by 3 cm. This pouching of the aorta was made up of the outer coats of the artery and was filled with an old, lamellated, partly organized thrombotic mass. The large branches of the aorta were all thickened and tortuous, especially the renal and splenic arteries which were bony hard. The splenic artery turned upon itself so that it formed a "snail shell" structure. The mesenteric arteries showed very slight atherosclerotic thickening but no occlusion. The inferior vena cava was normal. There was moderate atherosclerotic thickening of the smaller pulmonary vessels and a few grayish clots in the small arteries.

Pituitary: The organ was much smaller than normal.

Brain: Grossly normal. In the left occipital cortex there was a small area suggesting a mild scarring. All the vessels showed marked arteriosclerotic changes.

Amyloid was demonstrable in the kidneys, liver and spleen.

Microscopic Examination

Liver: Fatty change is seen in the liver cells, particularly in the periportal region. Slight congestion of central veins is present. Walls of arteries contain amyloid, as detected by Congo red stain.

Spleen: The follicles are not prominent. Arterioles of malpighian bodies are markedly thickened, the lumens narrowed. There is hyalinization of the walls of many of the vessels. The pulp shows a considerable degree of congestion and marked increase in

connective tissue. The malpighian arterioles contain amyloid and scattered throughout are streaks of amyloid.

Suprarenal: A considerable amount of lipoid accumulation in fascicular and glomerular layers is present. There is a large quantity of amyloid in the cortex, which in places extends beneath the endothelium of the capillaries and is accumulated in large quantities, obliterating the cellular structure of the parenchyma.

Heart: Extensive scar tissue formation in the myocardium with replacement fibrosis of muscle is present. Hypertrophy of muscle fibers is moderate. Section through the left ventricle in the region of the lamellated thrombus shows extensive fibrosis of myocardium, vacuolization of muscle fibers with hypertrophy of remaining cells. Marked fibrous thickening of endocardium is seen. Overlying the endocardium, and partly attached to it, is a pink-staining material containing large calcific plaques. Some degree of organization is present at the base of the thrombotic mass.

Kidney: The glomeruli are entirely replaced by a pink-staining, acellular, amorphous material. Ghosts of glomeruli are seen. Many of the glomeruli are small, irregular or completely destroyed with not a single intact glomerulus in the sections. There is a marked increase in interstitial connective tissue with a varying degree of atrophy of involved tubules. In some areas the tubules are dilated and filled with pink-staining material. In other areas they are reduced to a fine lumen and flattened epithelium. There are areas of round cell infiltration in the interstitial tissue. The arterioles are thickened, the lumens reduced in size, and the walls are replaced with a homogeneous material, which stains deeply with Congo red. Amyloid is found also in the medulla beneath the epithelium of the tubules.

Aorta: There is marked thickening of the intima with replacement fibrosis of connective tissue which is extensively hyalinized. The intima shows numerous cholesterol crystal spaces and areas of calcification which extend into the media.

Comment

Several interesting features are present in this case. The history was unusual in that the progressive loss of weight, asthenia, anorexia and abdominal pain suggested an intra-abdominal neoplasm. The X-ray of the chest was further misleading in that a localized arteriosclerotic aneurysm in the descending aorta simulated a primary

malignant growth. The hydrothorax requiring repeated thoracentesis, the gallop rhythm, the edema of the dependent parts and the hepatic enlargement fit into a picture of congestive heart failure associated with extensive myocardial infarction and are probably unrelated to the amyloid disease. It is noteworthy that the amyloid disease, as in Case 2, was practically limited to the kidneys and was sufficiently severe to cause uremia and a marked reduction of blood proteins simulating a picture of a nephrosis. There is no apparent etiological factor present, indicated either by the history or by the autopsy findings, for the amyloid disease.

DISCUSSION

The frequent association of the deposition of amyloid material in the various organs with chronic suppurative processes led to the conception that this metabolic disturbance is dependent on continued destruction of tissue protein. The nature of amyloid material was investigated by many workers.⁶ The protein nature of amyloid was pointed out by Friedreich and Kekulé⁷ in 1859 and substantiated by Kühne and Rudneff⁸ in 1865. It was found that amyloid organs contain chondroitin-sulphuric acid. On the basis of this observation Krawkow⁹ devised a method for the extraction of amyloid and found it to be a compound of protein with chondroitin-sulphuric acid. This view was contradicted by the work of Hanssen,¹⁰ who studied amyloid isolated mechanically from sago spleens in pure form, and found that this material contained no chondroitin-sulphuric acid, although the amyloid organs contained an excess of sulphur as sulphate. Eppinger¹¹ analyzed chemically the amyloid obtained from a solitary amyloid mass in the liver. He found that the dried material yielded no phosphorus and no sulphur but contained purines, diamino acids, much tyrosine and no carbohydrate. Of the amino acids present, glycocoll and phenylalanine, tyrosine, leucine and alanine comprised 42 per cent, arginine 14.67 per cent, tryptophane 4.41 per cent, glutamic acid and asparaginic acid 13.08 per cent. No cystine or histidine was present.

Experimentally it has been shown that the injection of bacteria such as staphylococci and various culture filtrates, and chemical agents such as sodium caseinate, may produce extensive amyloid disease in the spleen, liver or other organs of mice. Even the injection of turpentine with the production of a sterile abscess may call forth an amyloid reaction.

Kuczynski,¹² who succeeded in producing amyloid disease in mice by the injection of 5 per cent sodium caseinate solution, also found it could be produced by the feeding of proteins. These findings were confirmed by many investigators.

Letterer¹³ observed amyloid deposition in mice by the injection of egg-white, gelatin, nuclein, zein, peptone, casein-peptone and Witte's peptone. He concluded that many parenterally injected proteins may give rise to amyloid. He further found that amyloid disease could be produced in a few days by implantation of a piece of sterile mouse spleen or liver into the peritoneum.

The ease of production of amyloid disease in mice and the frequent spontaneous occurrence in this animal, as mentioned by Wells,¹⁴ cast some doubt on the validity of the conclusions drawn from such experimental studies.

Considerable controversy has arisen concerning the reversibility of amyloid disease. In view of the cases reported, both in this and in other communications of amyloid unassociated with apparent suppuration, this question is of considerable importance. Leupold⁶ demonstrated that amyloid deposition may be a reversible process. Kuczynski¹² believed that amyloid may be digested by the endothelial cells of the liver. He found these cells in mice filled with amyloid after the injections of the caseinate used to produce this process had been discontinued. Morgenstern¹⁵ confirmed this observation in amyloidosis produced by parenteral injections of albumin. He produced typical amyloidosis in 25 to 50 days in white mice. In other studies Morgenstern¹⁵ gave nutrose to white mice for 30 to 40 days and confirmed the presence of amyloid in the liver by biopsy. Biopsies were again taken 2 weeks after the injections were discontinued. After many months the animals were killed. The amyloid showed marked regression after 2 months. The deposits were surrounded by a granulomatous tissue containing fibroblasts, occasional giant cells and newly formed capillaries. Much less amyloid was apparent after 3 months. Four months after the injections had been discontinued complete resorption and disappearance of the amyloid occurred in some cases. He concluded that if the cause of the amyloid disappears, the amyloid deposits will disappear also.

Dantchakow¹⁶ also observed the absorption of amyloid in mice 2 months after discontinuing the injection of staphylococci. She

noted, however, that if the amyloid deposits were considerable, they were still present 6 months after the injections were discontinued, and she believed that amyloid was not resorbed if present in large amount.

It is conceivable that in the cases reported in this communication in which no apparent source of suppuration had been found that some previous suppurative process initiated the disturbance in protein metabolism. This, however, would seem unlikely. There have now been reported in the literature at least 6 other cases of diffuse amyloidosis in the absence of any suppurative process. It would seem likely that under certain conditions a fundamental disturbance in protein metabolism may occur which results in this abnormal deposition of an unusual protein. It would be interesting to investigate whether the diet plays any rôle in such a disturbance in human beings.

SUMMARY AND CONCLUSIONS

Three unusual cases of amyloid disease are reported in which an etiological factor was not demonstrated. The first was a female, 53 years of age, with extensive amyloid disease of the heart, tongue, gastro-intestinal tract and other organs, who died of congestive heart failure. The second case was a female, 16 years of age, with extensive amyloid deposits in the kidneys, liver and suprarenals, who died of uremia. She had an ankylosis of one joint without any evidence of suppuration. The third case was a female, 63 years of age, with amyloid contracted kidneys, who eventually died in uremia. She had a severe coronary sclerosis with an old occlusion of the descending branch of the left coronary artery and a healed infarction of the left ventricle. The amyloid disease was limited to the kidneys.

In view of the absence of any apparent suppuration it is suggested that this peculiar disturbance in protein metabolism may be independent of tissue destruction.

Addenda: After this article had been completed, Budd reported a case of primary amyloid disease of the heart in the *American Journal of Pathology*, 1934, 10, 299. In this case there was an extensive carcinoma of the prostate with extension into the bladder and surrounding tissues, a pyonephrosis and an acute endocarditis.

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DESCRIPTION OF PLATES

PLATE 10

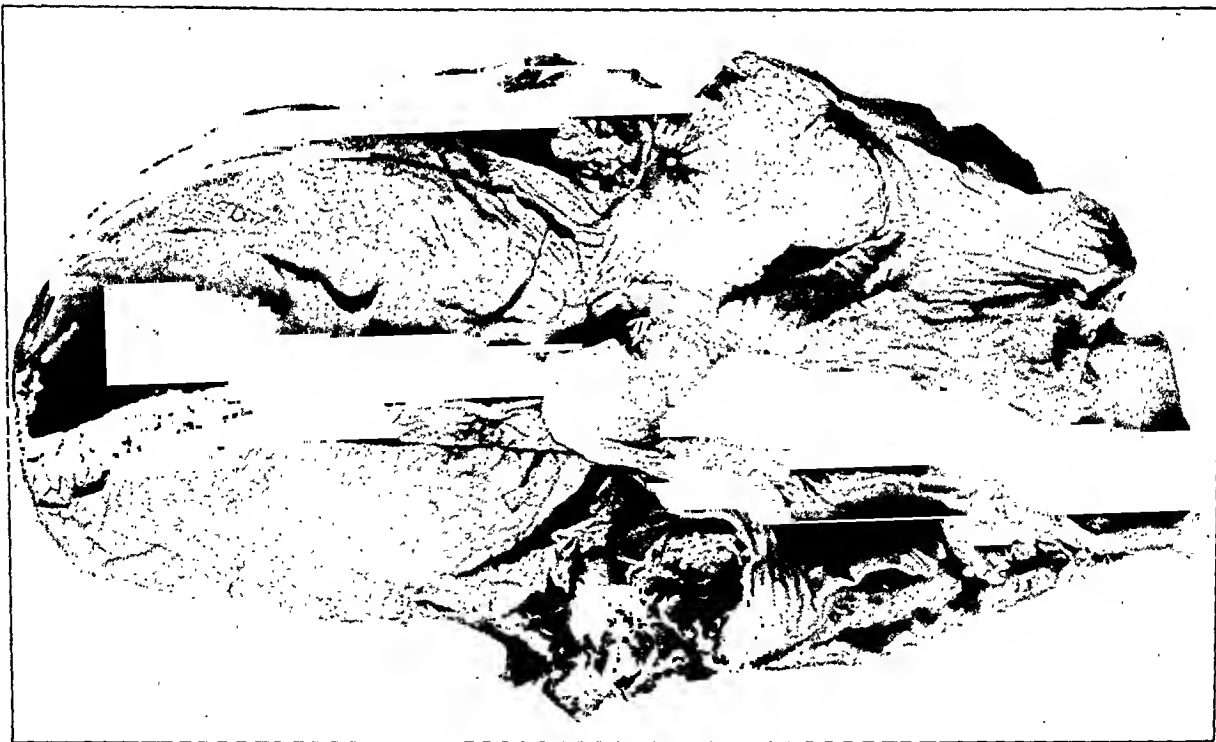
- FIG. 1. Case 1. Kidney. Cut surface showing narrow indistinct cortex and waxy appearance.
- FIG. 2. Case 1. External surface of kidney showing contraction and coarsely granular surface.
- FIG. 3. Case 1. Tongue, showing macroglossia.



1



2



3

PLATE II

FIG. 4. Case 1. Microscopic section of the heart showing amyloid infiltration.
X 240.

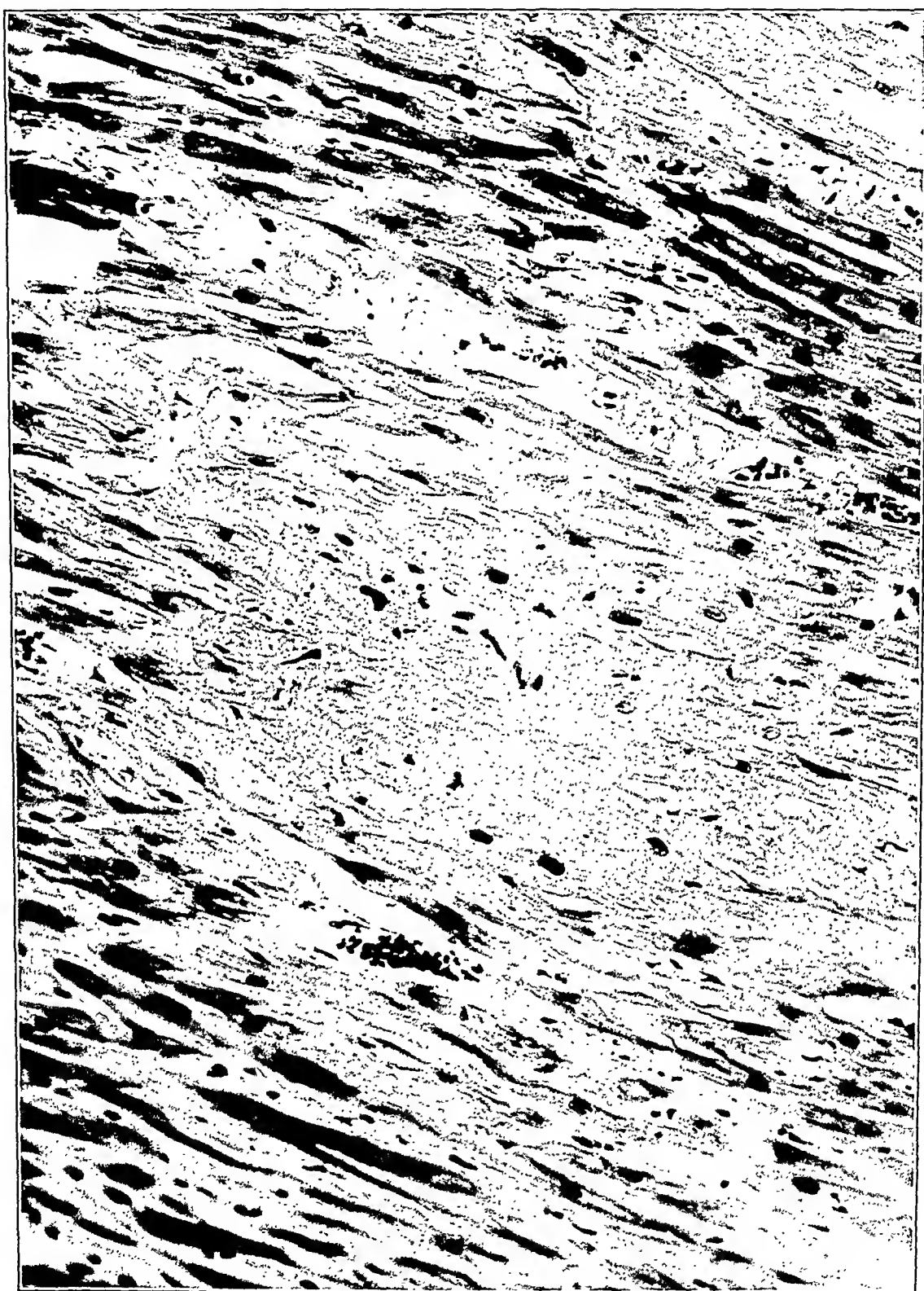
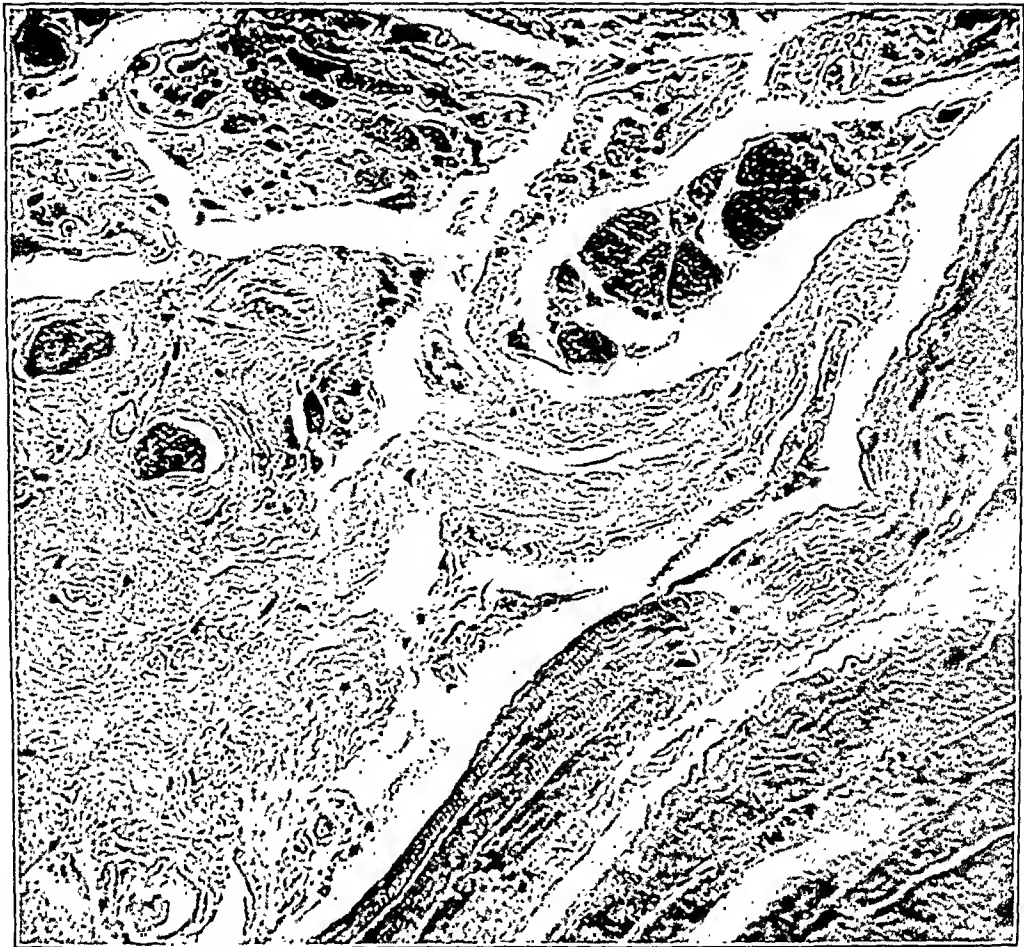


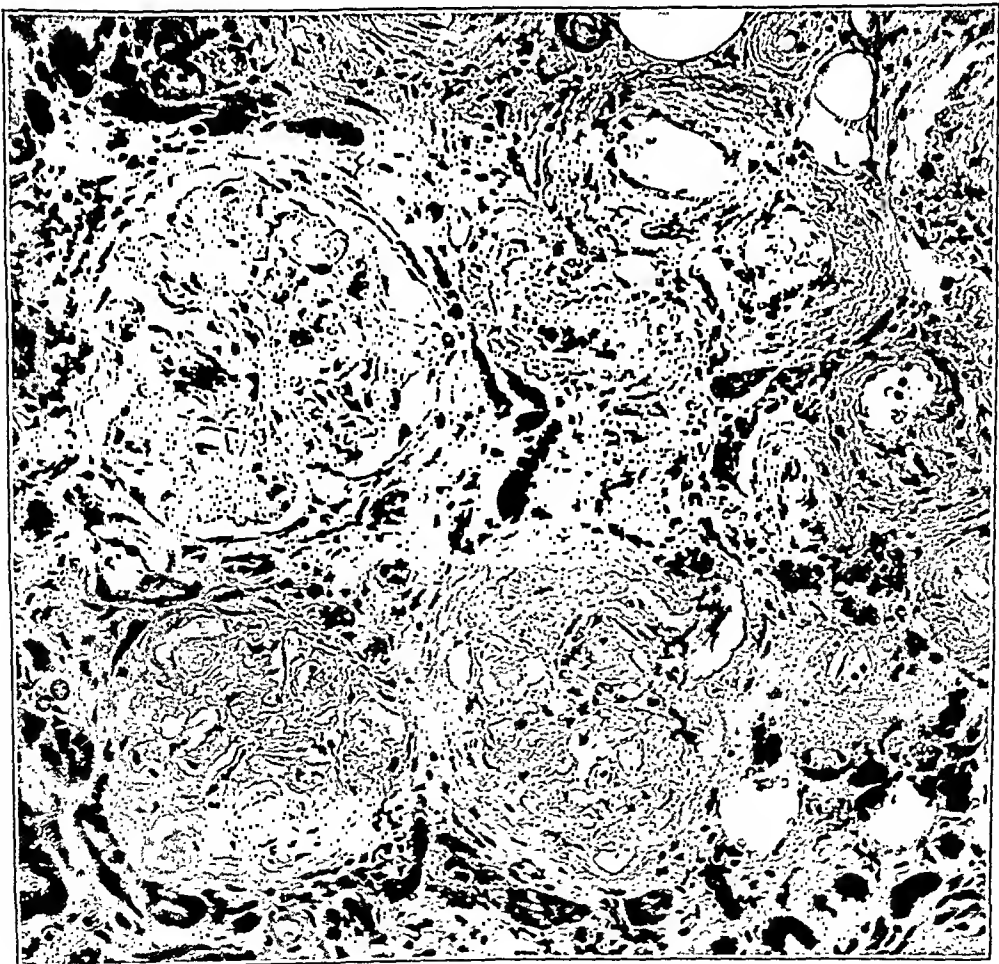
PLATE 12

FIG. 5. Case 1. Microscopic section of the tongue showing amyloid infiltration of the muscle. $\times 240$.

FIG. 6. Case 2. Microscopic section of the kidney showing severe amyloid infiltration. $\times 240$.



5



6

SUBACUTE LYMPHATIC LEUKEMIA *

HISTOGENETIC STUDY OF A CASE WITH THREE BIOPSIES

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The problem of the histogenesis of lymphatic leukemia still has many unsettled points. Therefore, it seemed of interest to follow the development of a case of lymphatic leukemia from the earliest stage. The pathologist usually has the opportunity to examine only material from advanced stages showing the pathological changes at the moment of death. We had the opportunity to observe a case of subacute lymphatic leukemia and to obtain biopsied inguinal lymph nodes at three different periods during the progress of the disease. These nodes show different stages in the development of the histopathology that is so characteristic of the postmortem material. They also explain important changes in the blood picture which occurred during the progress of the disease.

The hematopoietic activity of the reticulum was repeatedly emphasized by Maximow,¹ Downey and Weidenreich,² Klemperer,³ and others, and recent literature gives increasing data regarding the participation of the reticulum in the leukemic process. The best evidence for this is in the so-called leukemic reticulo-endotheliosis, or monocytic leukemia of the Schilling type. Fineman,⁴ Tice and Jaffé,⁵ and others also claim that this occurs in other types of leukemia. However, the postmortem material rarely shows any evidence to support this claim. Fineman's⁴ observations were made on biopsy material and he obtained good evidence for the derivation of the immature lymphocytes from the reticulum in a case of subacute lymphatic leukemia. The three biopsies and the postmortem material of the case which is reported here seemed to furnish ideal material for the further study of this question.

* Received for publication July 16, 1934.

REPORT OF CASE *

Clinical History: The patient was a white male, 6 years of age. The history obtained from the parents on admission was as follows. Chief complaints were pallor, weakness, epistaxis, and generalized lymphadenopathy of 6 months duration. In July, 1933, bleeding from the left nostril occurred, continuing for 3 days. He was studied in another hospital a few weeks prior to admission and the diagnosis of lymphatic leukemia was made. Three deep X-ray treatments were given, the white cell count varying in the meanwhile from 40,000 to 21,000. Three days prior to admission to the University Hospital epistaxis began again and the patient was admitted in an extremely dehydrated condition.

Physical examination revealed a very anemic boy with marked dyspnea. Both ears showed a profuse, purulent discharge. The neck showed bilateral cervical adenopathy. The chest organs were without abnormalities. The spleen was markedly enlarged, extending below the costal margin. The liver was not felt.

Blood Examination: On admission the hemoglobin was less than 10 per cent, red blood cells 800,000, white blood cells 10,000. The blood smear disclosed a marked anisocytosis, poikilocytosis and hypochromasia. The neutrophil leukocytes showed marked toxic changes. There were occasional immature lymphocytes and large reticulo-endothelial cells.

The patient received seven blood transfusions within the next 14 days and responded with a marked improvement, the hemoglobin rising to 51 per cent.

Because the blood examinations revealed many large reticulo-endothelial cells in addition to the mature and immature lymphocytes, a right inguinal lymph node was removed on Jan. 6, 1934, for the purpose of clearing up the diagnosis. The lymph node was enlarged. The imprint preparation of the node showed the presence of immature lymphocytes, and sections revealed a marked hyperplasia of the reticulum.

During the following 2 months the patient was able to be up and around and seemed to be experiencing a remission. The white cell count ranged around 35,000, with 96 per cent lymphocytes, the majority of which were of the immature type. The blood picture therefore indicated a diagnosis of lymphatic leukemia, while the lymph node of the first biopsy material revealed only a marked hyperplasia of the reticulum.

On Feb. 7, 1934, a second biopsy of a left inguinal lymph node was performed. A markedly enlarged node measuring 2 by 1.5 by 1 cm., hard, with a pinkish gray transparent cut surface was removed and examined. Feb. 27, 1934, a third biopsy was performed, with essentially the same findings. A few weeks later the patient became very weak, the temperature rising to 105°. He also developed eruptions over the entire body. Additional blood transfusions were given without results and the patient died quite suddenly March 8, 1934.

POSTMORTEM EXAMINATION

The report of the findings is restricted to the pathological changes. Autopsy was performed 15 minutes after death.

* For the case report and the use of material we are greatly indebted to Professor I. McQuarrie, Head of the Department of Pediatrics, University Hospital.

The body is that of a poorly developed, undernourished white male, 6 years of age. The skin is markedly anemic. Multiple petechiae are present on the trunk, but are most numerous on the back. There is a slight discharge in the left ear. The liver is markedly enlarged, reaching almost to the iliac crest. The heart is slightly enlarged. The muscles of both ventricles are flabby. Multiple small nodules are scattered in the lungs. The spleen weighs 440 gm., is quite hard and shows indistinct follicles. The liver weighs 1425 gm., is hard, and on the brownish red cut surface numerous pin-head-sized yellowish areas are seen. Generalized lymphadenopathy is present. Both kidneys are pale and flabby with numerous petechiae.

Anatomical Diagnoses: Edema of lungs, subacute lymphatic leukemia, bilateral otitis media suppurativa.

MICROSCOPIC EXAMINATION

The liver presents massive subcapsular and portobiliary infiltration of mononuclear cells with basophilic cytoplasm. The sinusoids are relatively free of the infiltration. In the spleen the malpighian bodies are markedly enlarged; some areas are normal, but most of the organ shows complete obliteration of the structure with an overgrowth of cells. The lymph nodes in the mesenteric region present uniformly dense masses and branching cords of cells. The kidneys are extensively infiltrated. The lungs show marked edema. There is a small area of peribronchial infiltration.

STUDY OF THE BLOOD

Blood examination on admission showed hemoglobin less than 10 per cent, red blood cells 800,000, white blood cells 10,000. The differential count was eosinophils 0 per cent, basophils 0 per cent, band forms 0 per cent, segmented cells 35 per cent, lymphocytes 58 per cent, monocytes 0 per cent, reticulo-endothelial cells 7 per cent. Marked anisocytosis, poikilocytosis and hypochromasia were present. The segmented leukocytes showed marked toxic changes. Daily examination of the blood revealed the presence of large reticulo-endothelial cells and some immature lymphocytes. These large reticulo-endothelial cells in smears stained with May-Grünwald-Giemsa were characterized by abundant, pale bluish cytoplasm containing some yellowish hyaloplasm in very small vacuoles, and a

relatively small nucleus with a fine and regular chromatin network and distinct parachromatin, with more or less indistinct nucleolus. In many instances the leptochromatic nuclear pattern of these large cells had become coarser, so that the nucleus resembled the nucleus of a lymphocyte, while the cytoplasm was still of reticulo-endothelial type (Figs. 1, 2 and 3). Cells having a distinct lymphocytic nucleus, but with cytoplasm of reticulo-endothelial type like the cell shown in Figure 4, were also found.

Sixty to 85 per cent of the lymphocytes were of immature type. These immature lymphocytes, when stained with May-Grünwald-Giemsa (Pappenheim), were characterized by a large nucleus and a small amount of definitely basophilic bluish cytoplasm. The nucleus presented an extremely fine and regular chromatin network and an abundant and distinct parachromatin, which was composed of numerous, fine, minute rounded granules embedded in a continuous mass of chromatin (leptochromatic structure, as shown in Fig. 5). These immature cells often possessed definite nucleoli. The immaturity in many instances was so marked that it was not possible to tell the direction of the differentiation. Occasionally the presence of immature cells with very fine nuclear pattern, having one or two distinct nucleoli and definitely basophilic cytoplasm (lymphoidocyte of Pappenheim, myeloblast of Nägeli) was noted. Similar cells having azure granules in the bluish cytoplasm and possessing a leptochromatic nuclear pattern, but without any definite nucleoli (leukoblasts of Pappenheim), were also present.

Numerous normal lymphocytes were always present. However, many of the mature lymphocytes presented atypical features, such as abundant cytoplasm, staining a very pale or deep bluish color, and a nucleus with rather heavily condensed chromatin masses (plasma cells). Occasionally nucleated red cells were present.

STUDY OF THE IMPRINT PREPARATIONS OF THE FIRST BIOPSIED LYMPH NODE

With imprint preparations tissue cells are studied under the same conditions and with the same technique as that used for blood smears. Blood cells are usually studied in smears where they are spread out in a thin film, while tissue cells are studied in sections where they preserve their original stereometric form. It is therefore

difficult to compare the cells of sections with those of the blood smears, and this might explain those numerous incongruities in the comparison of peripheral blood cells with the tissue cells. To demonstrate any transitional forms between certain types of tissue cells and cells of the peripheral blood, one will need good imprint preparations. The technique of these imprint preparations is briefly as follows. The freshly cut surface of a small piece of tissue is touched very gently to a perfectly cleaned slide, without any pressure or smearing. The slides are dried rapidly by whipping through the air and shrinkage is thus avoided. The May-Grünwald-Giemsa staining technique will give the best results in $1\frac{1}{2}$ or double concentration of Giemsa's stain because of the large number of cells. Such preparations were made from all three biopsied nodes and from the autopsied material.

Microscopic examination of the imprint preparations of the first biopsied node shows a few lymphocytes with narrow cytoplasm and a large leptochromatic nucleus having definite nucleoli. Numerous, large, reticulo-endothelial cells similar to those described in the peripheral blood are also present. The immature lymphocytes in many instances contain vacuoles in the cytoplasm and some show sharp indentations of the nuclear membrane.

STUDY OF IMPRINT PREPARATIONS FROM THE SECOND AND THIRD BIOPSIED LYMPH NODES

These preparations present many immature lymphocytes (Fig. 6) which have the same leptochromatic nuclear pattern and narrow cytoplasm as those seen in the blood smears (Fig. 5).

DESCRIPTION OF THE MICROSCOPIC FINDINGS OF THE LYMPH NODE FROM THE FIRST BIOPSY

The node from the inguinal region was enlarged, measuring 1.5 by 1 by 0.5 cm. It was soft and dark grayish pink in color. The cut surface was uniformly grayish pink and showed minute hemorrhagic areas.

Microscopic examination shows that the normal structure of the lymph node is entirely obliterated; the entire structure is looser and the sharp difference between cortical and medullary portions

has disappeared. In the cortical portion only one or two follicles are recognizable; other follicles have entirely disappeared. There are places, however, where only small conglomerations of lymphocytes represent the remnants of follicles. The predominating type of cells shows syncytial arrangement (Fig. 7). These cells are characterized by a long oval and slightly acidophilic cytoplasm having an oval nucleus with little chromatin (Fig. 8), and seem to be attached to a syncytial network. There are places, however, where the attachment is looser and the cells are more rounded and have a more basophilic cytoplasm. Between these cells there are numerous, free round cells with markedly basophilic cytoplasm and heavily stained nucleus with conspicuous nucleoli. These cells are probably immature lymphocytes. The sinuses in the cortical portion are widened and filled with red cells and lymphocytes.

DESCRIPTION OF THE NODE FROM THE SECOND BIOPSY

This biopsy was performed 4 weeks after the first biopsy. A markedly enlarged node measuring 2 by 1.5 by 1 cm. was removed from the inguinal region. It was soft and grayish red in color. The grayish pink cut surface was uniformly transparent.

Microscopic examination reveals a node that is extremely rich in cellular elements, especially in the medullary portion. The normal structure is entirely obliterated. The sinuses and intersinusoidal spaces are crowded with a dense mass of cells. These cells are characterized by a relatively large basophilic cytoplasm and a large nucleus with a dense chromatin network and are no longer arranged in a syncytial network. They seem to be immature leukemic lymphocytes.

In the cortical portion there are still fairly well defined follicles with light germinal centers containing numerous, large, oval reticular cells with pale nuclei, and scattered between these are many large lymphocytes with heavy chromatin particles and a conspicuous nucleolus. The peripheral portion of these follicles is composed of small dark lymphocytes, which are sharply demarcated from the surrounding dense mass of immature leukemic cells. Figure 9 is taken from a well preserved cortical germinal center. It shows clearly that the central portion is composed mainly of large reticular cells and that the narrow marginal portion is composed of small

lymphocytes with dark nucleoli which separate the germinal center cells from the leukemic lymphocytes. Except for the narrow marginal band of small lymphocytes the follicle and its germ center is of normal structure, and there is no evidence to indicate that the immature leukemic lymphocytes located in the interfollicular and medullary portion of the node have originated from the normal lymphocytes of the follicles or from lymphoblastic germ center cells, as claimed by many authors.

DISCUSSION

Despite the mass of literature on the subject, the origin of lymphocytes in postfetal life is still a debated question. According to Maximow,¹ Danchakoff,⁶ Weidenreich,⁷ and Thiel and Downey,⁸ the mesenchymal syncytium gives rise during embryonic life to all the different blood cells, including the lymphocytes. There are many different views concerning the regeneration of the lymphocytes in postfetal life. Helly,⁹ Nägeli,¹⁰ and others, maintained that regeneration was homoplastic, meaning that lymphocytes give rise to other lymphocytes. Weidenreich,⁷ Downey and Weidenreich,² Maximow,¹ and others believed in the heteroplastic form of regeneration, claiming that the fixed cell can give rise to lymphocytes throughout life. Concerning this fixed cell there are also quite different views expressed by different authors. While Downey and Weidenreich² spoke of reticulum, which possesses universal potencies, Marchand¹¹ claimed that the periadventitial cells have embryonic potencies.

The localization of lymphocytic regeneration is still a point of disagreement. Flemming¹² regarded the clear centers of the follicles as germ centers on account of the numerous mitotic figures which they contained. Maximow¹ spoke also of the resting and active phase of germ centers. Flemming's theory was opposed by Marchand,¹¹ who pointed out the sharp demarcation line between the light central zone and the dark marginal portion and claimed that there are, therefore, no transitional forms between the small lymphocytes and the large cells. Hellman,¹³ in a series of papers, urged against the Flemming theory that there is a quantitative disharmony between the light central zone and the dark marginal portion. One can frequently observe large germ centers with numerous mitotic figures

and very small or no marginal portion. He emphasized the fact that in cases of lymphatic leukemia with enormous lymphocytic production the light areas of the follicles have disappeared, while in certain infectious conditions there is a marked increase of germ centers without simultaneous increase of lymphocytes in the peripheral blood. Heiberg¹⁴ pointed out that the germ centers often contain numerous pyknotic nuclei and evidences of phagocytosis (tingible bodies). Most authors believe that the germ center has a double function, regeneration and defense. Under normal conditions the germ center gives rise to lymphocytes, but under the influence of excessive demands the supply of lymphocytes in the normal sites of their formation may become exhausted. This leads to heteroplastic formation of lymphocytes from the tissue which retained its embryonic potencies.

It is known that a uniform syncytial structure characterizes the embryonic lymphatic tissue, which in the early development consists only of mesenchymal cells. Maximow,¹ Downey,² Marchand¹¹ and Klemperer³ emphasized the morphological similarity between embryonic and adult reticulum, which suggests the possibility that the reticulum may still retain the early embryonic potencies. Maximow¹ distinguished between undifferentiated mesenchymal cells and reticulo-endothelial cells, which latter he considered as more or less differentiated cells. However, this distinction was on a functional basis, because there are no sharp morphological differences. Marchand¹¹ believed the periadventitial cells had universal potencies, while Herzog¹⁵ recently divided them into two groups of perivascular cells, one of which was already differentiated along the histiocytic line (Marchand's clasmatoocytes), while the other one is still multipotent (Zimmermann's pericytes, Maximow's perivascular mesenchymal cells). Von Möllendorff¹⁶ and his pupils claim to have proved that the fibrocytes in syncytial arrangement in the loose connective tissue still have universal potencies. Klemperer³ in 1932 considered that the undifferentiated mesenchyme of the adult organism includes the fixed "cytoplasmic reticulum" of the myeloid and lymphoid tissue, and the perivascular cells of Marchand-Herzog. He also agreed with Maximow that the "cytoplasmic reticulum" is not absolutely identical, either morphologically or functionally, with the reticulo-endothelial system of Aschoff-Kiyono, which latter is already differentiated in the phagocytic direction.

The adult organism retains a multipotent tissue, which is able to give rise to any of the blood cells. This heteroplastic blood cell formation must become logically evident in leukemias where there is the most excessive demand for blood cell formation. Lymphocytic regeneration in physiological conditions is still a debated question. Similarly, there is quite a disagreement regarding the sources of the greatly increased lymphocytes in lymphatic leukemia. Schridde¹⁷ maintained that the disappearance of the germinal centers was due to the overgrowth of germ center cells. Nägeli¹⁰ pointed out the fact, as a proof of the dualistic nature of the blood cells, that in myelogenous leukemia the process always starts outside of the germ centers. However, in experimentally induced extramedullary myelopoiesis, Dominici,¹⁸ Bloom,¹⁹ and Lang²⁰ showed definite evidence that myeloid transformation may start right in the centrum of the germ centers, suggesting that the omnipotent tissue is quite uniformly distributed in germ centers as well as in the pulp. Thiel and Downey,⁸ Mollier²¹ and Ono²² described the development of vascular and lymph sinuses within the reticulum of spleen and lymph nodes and proved that the flat sinus endothelial cells are direct descendants of the mesenchymal syncytium and that they retain their hematopoietic potencies.

Maximow¹ gave evidence regarding the close relation of the free stem cells to the fixed reticulum cells. Ewald²³ in a case of acute leukemia, in which 95 per cent of the white blood cells were more immature than the myeloblast, found a generalized hyperplasia of the reticulum cells, and the desquamation of these immature cells was also noted. Fineman⁴ obtained good evidence for the derivation of the immature lymphocytes from the reticulum in a case of subacute lymphatic leukemia. Rössle²⁴ reported chronic lymphatic leukemia without any involvement of lymph nodes, but with a generalized leukemic infiltration of the skin, where the hyperplastic reticulum was believed to give rise to the lymphocytes. Ungar²⁵ also observed a case of aleukemic lymphocytic reticulo-endotheliosis in which there was evidence for the origin of lymphocytes from the fixed reticulum. Recent careful studies of numerous cases of monocytic leukemia and of leukemic reticulo-endotheliosis clearly indicate the hematopoietic activities of the reticulum cells in the Schilling type, as well as in the Nägeli type. Schwarz,²⁶ Hittmair²⁷ and many others also felt that the mesenchymal tissue

of the adult organism is able to respond to certain forms of stimulation with a marked mesenchymal cell production. Klemperer³ recently claimed that not only in leukemias, but also in other pathological conditions (cirrhosis of the liver, Gaucher's disease), can the derivation of hemocytoblast from the generalized syncytial reticulum be observed. Tice and Jaffé⁵ found in their studies of the histogenesis of leukemias that the leukemic involvement, especially in stem cell leukemias, always starts in the medullary portion of the lymph nodes.

COMMENT

The case herein reported presented a marked hyperplasia of the reticulum in the biopsied lymph node from the earlier stage of the leukemic involvement. At the same time, in the peripheral blood there were quite a number of large reticulo-endothelial cells in addition to immature lymphocytes. In many instances these reticulo-endothelial cells showed a nucleus with a rather dense chromatin structure resembling a nucleus of a lymphocyte (Figs. 1, 2 and 3). In a later stage the second biopsy material showed that the medullary portion was packed with a dense mass of large immature lymphocytes, while the cortical region showed more or less well preserved germ centers (Fig. 9). The subsequent disappearance of the large reticulo-endothelial cells from the peripheral blood was also noted. It must be considered, therefore, that the first involvement of the leukemic process begins with a diffuse proliferation of the reticulum. Klemperer³ stated that the cytoplasmic reticulum, which is normally neutrophilic or slightly acidophilic, becomes more basophilic during its hematopoietic activities, and that the basophilia appears first in the perinuclear zone. The chromatin structure also becomes more condensed. That was also noted in our biopsy material stained with methyl green-pyronin (Unna-Pappenheim). We found numerous transitional forms from reticulo-endothelial cells to large lymphocytes in the imprint preparations and in the peripheral blood from the earlier stages, which later disappeared. In a later stage the medullary portion was entirely replaced by a dense mass of large cells with basophilic cytoplasm. It was only in the cortical region where a few more or less well preserved follicles were retained. Simultaneously in the peripheral blood there was a marked increase of immature lymphocytes. This

finding again indicates that in the lymphoid tissue the mesenchymal syncytium is rather uniformly distributed and that lymphocytopoiesis is not restricted to the germinal centers or to preformed germ center cells (lymphoblasts of the dualists).

The evidence of extramedullary myelopoiesis in the medulla, as well as in germinal centers, the marked monocytic production in cases of leukemic reticulo-endotheliosis and finally the transformation of the mesenchymal syncytial cells into lymphocytes, indicate the embryonic hematopoietic potency of the syncytial reticulum cells.

SUMMARY

With three biopsies, taken at different times, the histogenetic development of a case of subacute lymphatic leukemia was followed.

In the early stages the lymph node from the inguinal region showed a diffuse hyperplasia of the syncytial reticulum cells. Many of these cells showed a beginning basophilia in the cytoplasm. At the same time the peripheral blood contained a number of larger cells with a cytoplasm that is characteristic of the reticulo-endothelial cell, but with a nucleus of lymphocytic pattern, indicating the heteroplastic origin of lymphocytes from the reticulum.

The later biopsy material from an inguinal lymph node presented a dense mass of immature lymphocytes in the medullary region, while the cortical germ centers were still preserved. The immature lymphocytes, therefore, originate in the medulla rather than in the germinal center of the follicles, and the diffusely distributed syncytial reticulum is the mother tissue for these immature lymphocytes.

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DESCRIPTION OF PLATES

PLATE 13

- FIGS. 1, 2 and 3. *Large reticulo-endothelial cells of the peripheral blood. May-Grünwald-Giemsa's stain. Note the abundant histiocytic cytoplasm and the slightly lymphocytic nuclear pattern. $\times 1800$.*
- FIG. 4. *"Transitional" cell of the peripheral blood. The nucleus is distinctly lymphocytic, while the cytoplasm is still histiocytic. $\times 1800$.*
- FIG. 5. *Immature lymphocytes of the peripheral blood. Note the small amount of cytoplasm and the relatively large nucleus with leptochromatic pattern. $\times 2400$.*
- FIG. 6. *Immature lymphocytes seen in imprint preparations from the second biopsied inguinal lymph node. Note the leptochromatic nucleus and small amount of cytoplasm. $\times 1400$.*

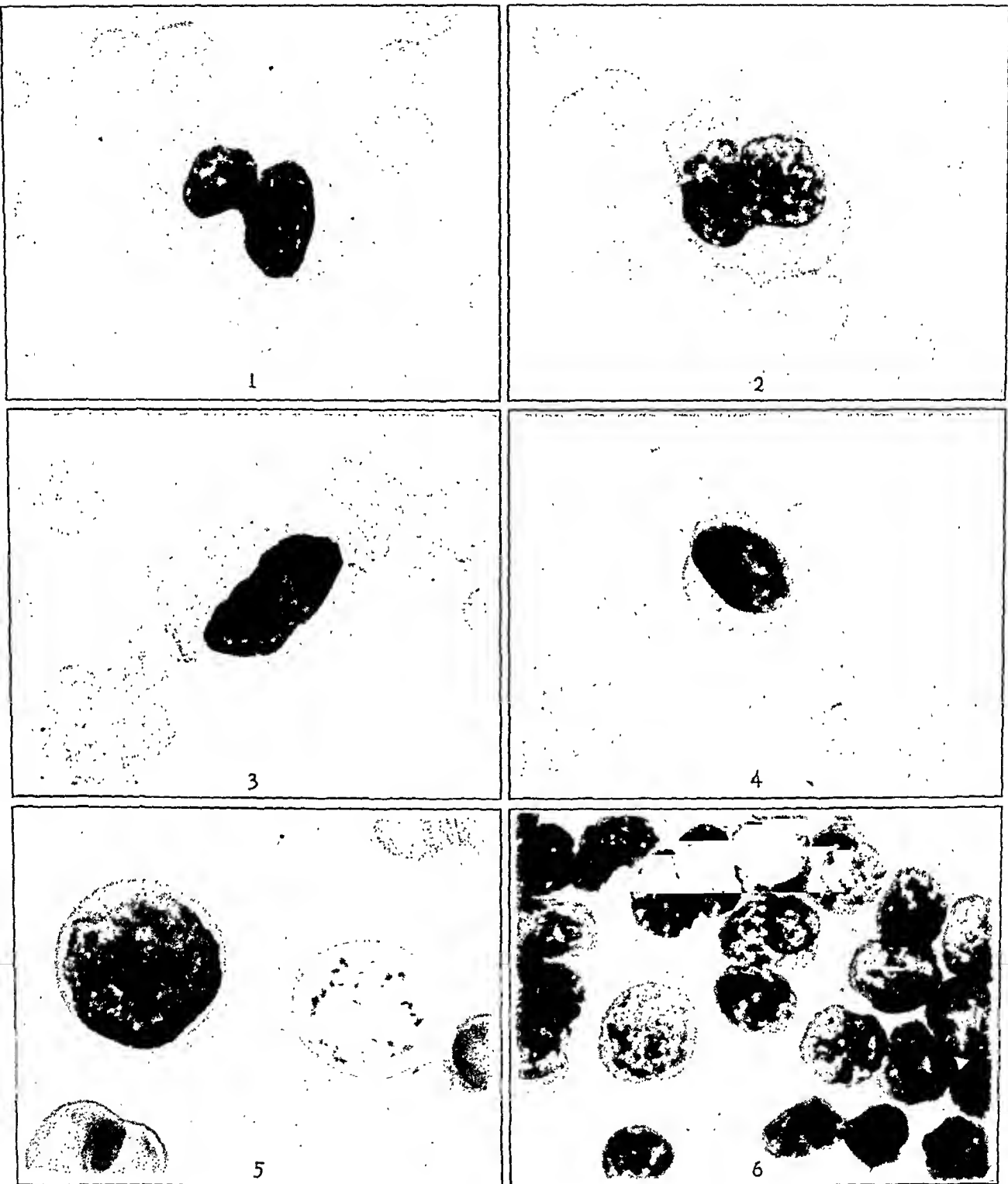
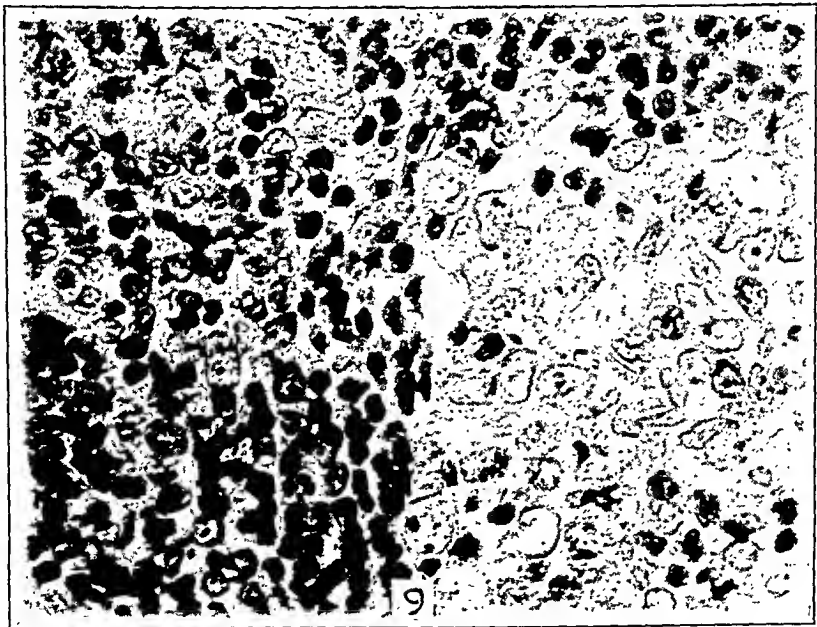
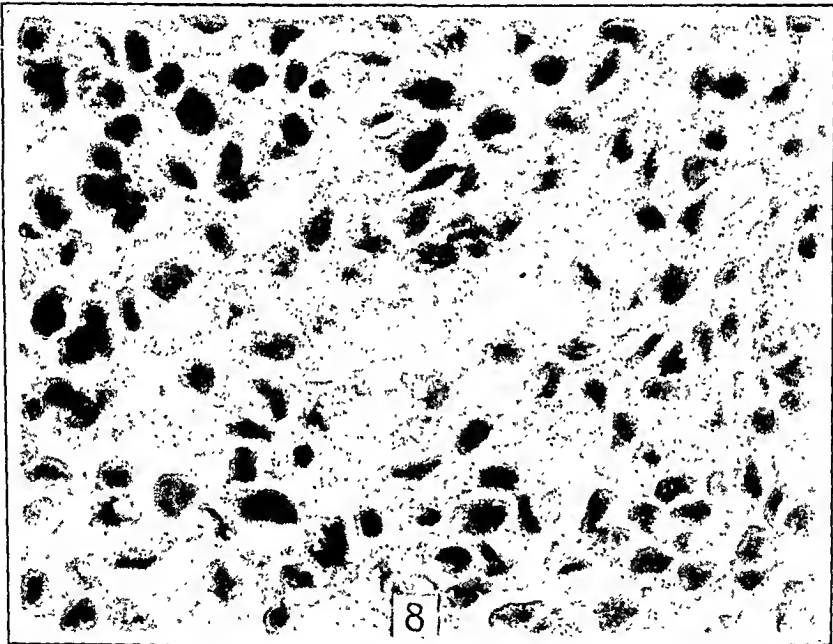
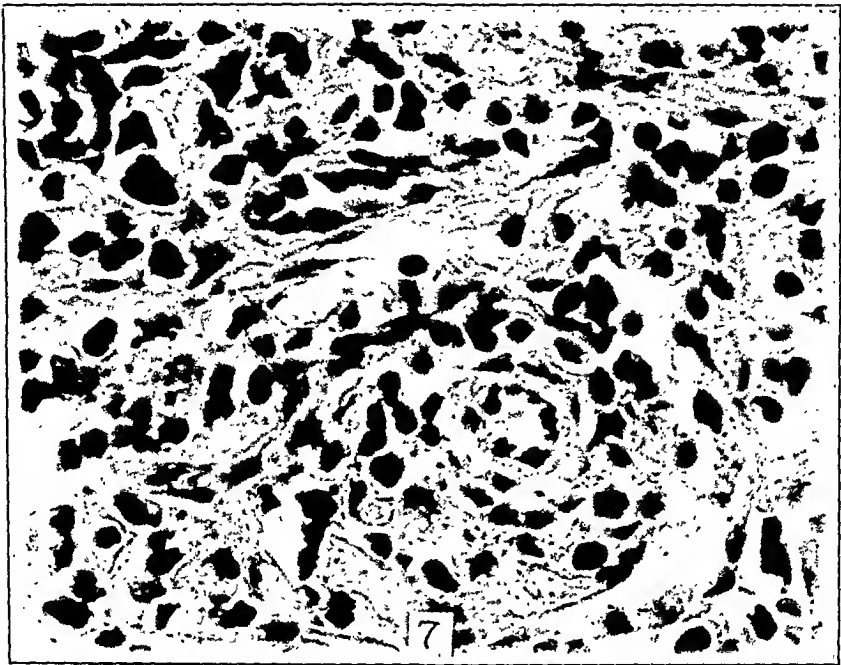


PLATE 14

- FIG. 7. Lymph node from the first biopsy. The normal structure is replaced by cells in reticular arrangement. Unna-Pappenheim's stain. $\times 960$.
- FIG. 8. Lymph node from the first biopsy. The high magnification shows the oval reticular cells with pale nucleoli. Unna-Pappenheim's stain. $\times 1800$.
- FIG. 9. Lymph node from the second biopsy showing the marginal portion of a follicle. The light area indicates the germinal center, which is composed mainly of large reticular cells. A small zone of deeply staining, small lymphocytes separates the germinal center cells from the leukemic cells Dominici's stain. $\times 1800$.



HEPATO-ADRENAL NECROSIS WITH INTRANUCLEAR INCLUSION BODIES *

REPORT OF A CASE

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The principal purpose of this communication is to present the description of intranuclear inclusion bodies in the parenchymal cells of the liver and adrenals of a 2 weeks old premature infant in whom the chief pathological findings were a widespread necrosis of the liver and focal cortical necrosis of the adrenals. This case was brought to my attention by Prof. S. Burt Wolbach, who had never observed similar histopathology in his wide experience in the study of diseases of infancy and childhood. So far as the writer is aware no disease of similar nature has been reported in the literature.

REPORT OF CASE

Clinical History: The patient was a 7 months premature female negro infant, 40 cm. in length, weighing 3 pounds. The mother and father were in good health and there was no history of miscarriages. The patient was the mother's first baby and was 16 hours old when admitted to the hospital.

Physical examination showed a drowsy infant who, when aroused, cried lustily. There was a large, soft, non-fluctuant swelling over the right side of the skull and the anterior and posterior fontanelles were normal. There was no jaundice. Her temperature was 97° F. The clinical diagnoses were prematurity and caput succedaneum.

During the first week in the hospital the patient voluntarily ingested increasing amounts of breast milk so that by the end of the week she was taking 35 calories per pound. The swelling over the parietal region of the skull slowly disappeared and she became less active. On the eleventh day of life it was necessary to give nourishment by gavage. Her temperature gradually rose to 100° F on the twelfth day. On the thirteenth day she was transfused with 30 cc. of citrated blood obtained from her father. Four hours later her diaper was stained with blood. A small amount of blood persisted in the feces and was visible in the food which she soon began to vomit. No unusual degree of jaundice was noted. Her temperature fell to 97° F, but she died about 12 hours after the transfusion, at 14 days of age. The discharge diagnoses were prematurity and caput succedaneum.

* Received for publication July 19, 1934.

AUTOPSY PROTOCOL

The autopsy was performed 3 hours after death.

Heart: The heart weighed 15 gm. The patent foramen ovale measured 5 mm. in diameter. A delicate row of pale red, small, granulation-like structures were found on the mitral valve.

Lungs: The right lung weighed 27 gm. and the left 22 gm. They were uniformly pink and normally crepitant except in the dependent portions where they were dark red in color and less crepitant than elsewhere. Cross-sections of the parenchyma showed numerous small red areas, which were interpreted as foci of hemorrhage rather than of pneumonic consolidation.

Spleen: The spleen weighed 10 gm., was dark red and firm.

Alimentary Tract: The stomach was small and contracted. There were a few small mucosal hemorrhages. The ileum and colon were normal.

Pancreas: Normal.

Liver: The liver weighed 75 gm. (birth weight of the liver of a normal full term infant 78 gm.). The enlargement seemed to be confined principally to the left lobe. The consistence was diminished and the surface mottled, there being numerous, irregular, grayish yellow to pale brown areas varying from less than 1 to 4 mm. in diameter. These were separated by broader zones of reddish brown parenchyma. The grayish yellow areas usually were discrete but in several instances were confluent. In the centers of several of these scattered regions were minute hemorrhagic spots. The lesions were not elevated above the plane of the surface of the liver. No exudate was present on the surface of the overlying capsule. The interior of the liver, exposed by sectioning, showed a fairly uniform distribution of the gross lesions throughout the parenchyma. No abscesses were visible.

Gall-Bladder: The gall-bladder was filled with pale brown bile less viscid than normal. Bile was expressed easily through the cystic and common ducts into the duodenum.

Kidneys: The right weighed 10 gm. and the left 9 gm. The fetal lobulations were prominent. The capsules were not adherent to the cortical substance. The cortex, medulla and pelvis of each organ were normal except for one small hemorrhagic area in the cortex of the left kidney.

Adrenals: The adrenals, which together weighed 4 gm., were normal.

Pelvic Organs: Normal.

Brain and Spinal Cord: The brain weighed 180 gm. and was considered to be normal at the time of removal. After fixation in formalin a note was made which stated that the substance of the brain was soft and fatty. The spinal cord was normal.

Bone Marrow: The bone marrow in the vertebral bodies was bright red in color.

Bacteriology: A culture of blood removed from the right auricle showed *Bacillus coli* and *Staphylococcus albus*. The same organisms were obtained from the peritoneal cavity by cultural methods.

MICROSCOPIC STUDY

Heart: There is a slight interstitial infiltration with polymorphonuclear leukocytes which is localized to the perivascular tissues. In rare instances the adjacent muscle fibers show minimal evidence of necrosis. No blocks were taken through the peculiar structures on the mitral valve. They may have been small "blood cysts," which are not uncommon in infancy.

Lungs: There is evidence of immature development. Many groups of alveoli are not fully distended. Other groups are over-distended and interalveolar septa often are ruptured. Occasional bronchi contain an acellular, granular, eosinophilic débris in which are numerous bacteria of variable morphology. This débris probably represents aspirated foreign material. In several alveolar spaces are extravasated red blood cells, an albuminous precipitate, asphyxial membranes and entrapped air. There is no inflammatory reaction.

Spleen: The follicles are small and of immature type and the sinusoids are distended with red blood cells. Small areas of hematopoiesis are composed largely of cells of the myeloid series. Numerous mononuclear phagocytes laden with hemosiderin are present. One small colony of cocci which seems to have stimulated no regional inflammatory reaction is found. Although the nucleoli of several of the lining cells of sinusoids are prominent there are no inclusion bodies similar to those which are found in the liver and adrenals.

Pancreas: Except for the presence of pancreatic tissue beneath the mucosa of the duodenum, no unusual histological findings are seen.

Ileum: Sections of the ileum disclose no lesions, but colonies of bacteria are seen in several vessels.

Kidneys: The only histological features of interest are those of immaturity and cloudy swelling and vacuolation of the cytoplasm of the convoluted tubules.

Urinary Bladder: No abnormal findings are noted.

Thymus: Normal.

Vertebra: No important changes are seen in the bone or bone marrow.

Brain and Spinal Cord: Blocks were taken from the cerebral cortex and the cerebellum but there are no lesions. The spinal cord shows a normal structure.

Liver: Blocks of liver were fixed in Zenker's fluid and formalin. Material which had been in formalin for 6 years was mordanted in Regaud's fluid for 48 hours. The tissue was embedded in paraffin and sections of from 5 to 7 microns in thickness were cut. The following stains and staining methods were used: hematoxylin-eosin, eosin-methylene blue, Wolbach's modification of the Giemsa stain, Gram-Weigert's method for the demonstration of bacteria, Ziehl-Neelson's carbol fuchsin stain for acid-fast organisms, Levaditi's technique for spirochetes, Mallory's anilin blue-acid fuchsin-orange G collagen stain, and Mallory's phosphotungstic acid hematoxylin.

All sections show an extensive acute necrosis with a few widely isolated clusters of viable liver cells remaining as a rule around the portal areas. The pale yellow areas which were noted in the gross specimen differ histologically from the intervening reddish brown tissue in that they represent zones where there is a most severe type of degeneration. In these regions the lobular architecture is indistinguishable. There are no continuous cords of liver cells and the sinusoidal system is disrupted. Remnants of liver cell cytoplasm, pyknotic fragments of nucleoplasm and degenerated cellular elements of the blood are fused into a conglomerate mass of débris which fills and obliterates the sinusoidal spaces and central veins. The portal structures often have succumbed, but they appear to be the last part of the structural unit to have undergone necrosis. Very little fibrin is present, although occasional necrotic vessels filled with remnants of fibrinous thrombi are found. There are no abscesses or significant local accumulations of leukocytes.

The friable reddish brown tissue which lies between the yellow

areas of necrosis shows a similar type of degeneration but the necrosis in these regions has not advanced to such a complete state of colliquation. The shadowy outlines of interrupted columns of liver cells separated by indistinct, though partly intact, sinusoids are visible amid the masses of cellular débris. Numerous extravasations of blood cells are present but most of the blood seems to be confined in the irregular, distorted sinusoidal spaces. Even though the major portion of the parenchyma is necrotic, the red blood cells appear to be much more viable in the reddish areas than in the yellow zones which have been described above. An occasional central vein is detected. These veins rarely are dilated or filled with fibrin networks in which partially degenerated blood cells are enmeshed. Portal structures and periportal connective tissue usually can be recognized but often they are involved by the necrotizing process, which seems to spread from the parenchyma, invade the periportal connective tissue and destroy, first the collagen, secondly the ducts, and finally the blood vessels. The portal veins often are dilated and, occasionally, when involved by the spread of the primary process, they are filled with fibrinous thrombi which blend at their margins with the indistinct outlines of the swollen, homogeneous, degenerated vascular wall. The reaction to this injury is very slight. The polymorphonuclear leukocytes are slightly increased in number but there is no proliferation of bile ducts or connective tissue cells.

Study of the widely separated small islands of viable liver cells and the gradual transition through various stages of degeneration in the zones bordering on the areas of necrosis discloses the most significant histological findings. These clusters of cells comprise about one-tenth of the total volume of the liver. They usually lie adjacent to the portal areas and in no instance is there a wholly intact lobule. As a rule, the architecture is almost normal, either in the center of the groups of cells or in that part which is in apposition to the periportal connective tissue. By arbitrary reconstruction of the process it would seem that the disease in the beginning must have affected the central and midzonal regions of the primary lobule.

The description of a typical viable remnant of hepatic parenchyma (Fig. 13), as studied in the sections fixed in Zenker's solution and stained by Wolbach's modification of the Giemsa stain, may suffice to exemplify a fairly uniform picture. The portion of this irregular, poorly demarcated island of cells which is adjacent to the periportal

connective tissue shows an orderly arrangement of liver cells and sinusoids. As one progresses toward the central veins an increasing number of structural alterations becomes apparent, until finally the disintegrating columns of abnormal liver cells and distorted sinusoids blend imperceptibly into the surrounding necrotic mass of liver structure.

The appearance of the individual liver cells, and especially their nuclei, is of principal interest in this study. In the periportal zone many cells are normal in size and shape. The cytoplasm is pale bluish pink, normal in structure, and the nuclei are round or oval. Delicate chromatin networks ramify throughout the nucleoplasm. The nucleoli usually are slightly eccentric in position. Among these cells, which are apparently normal, there are a few cells in which only the nuclei show abnormalities. In the first place the nuclear membranes are slightly irregular or undulate. Secondly, the chromatin networks are indistinct in the center of the nucleus and there is a definite tendency toward an accumulation of chromatin in the zone which lies adjacent to the nuclear membrane. Thirdly, most nucleoli are situated adjacent to the nuclear membrane. In these cells the relative homogeneity of normal cytoplasm is changed to a delicately granular or reticular substance. The granules are slightly acidophilic and are more deeply stained than the background of pale cytoplasm.

As one progresses from the relatively normal periportal zone of liver cells an increasing number of cytological abnormalities becomes apparent. Adjacent to the necrotic parenchyma almost all the cells exhibit the peculiar changes which characterize this malady. The unique histopathology is restricted to the nuclei, although as a rule there are attendant cytoplasmic changes of variable nature.

The nuclei for purposes of description may be divided into two groups: first, those in which there are acidophilic intranuclear bodies and, secondly, those that are characterized by abnormal basophilic intranuclear structures. A certain number of nuclei serve to exemplify the morphological and tinctorial gradations between these two principal groups.

The nuclei which contain acidophilic bodies are more numerous than the other types. The smallest bodies are found not infrequently in cells which otherwise appear to be normal. More commonly there are detectable cytoplasmic and nuclear changes. The cytoplasm

often is swollen, granular and delicately vacuolated. In the early stages the nuclei are appreciably enlarged, the chromatin networks are altered and the nucleoli are either eccentric in position or are in apposition to the nuclear membrane. In such cells minute pink granules of irregular contour appear between the partially disrupted strands of deeply basophilic chromatin which seems to be maintained distinctly apart from the acidophilic bodies (Fig. 1). The strands of chromatin gradually disappear in the center of the nucleus and preceded by the nucleolus the remnants of chromatin retreat toward the nuclear membrane, leaving a central area into which the acidophilic granules migrate (Fig. 2) so as to fuse eventually into a single, pale pink, amorphous, irregular mass of a slightly deeper red tint than the elementary bodies of which it is comprised (Fig. 3). Occasionally, heavy strands of chromatin retain their position and traverse the diameter of the nucleus in such a manner as to maintain barriers between the agglomerating unit bodies. In such instances two and rarely three distinct acidophilic masses become segregated in the divided zones (Fig. 4). As a rule a single amorphous mass occupies the center of the nucleus. As it condenses, it becomes more homogeneous and the nucleolus and chromatin retreat farther and farther so that eventually they become aligned along or intimately fused with the nuclear membrane (Fig. 5). During this stage the nucleus usually decreases in size and the nuclear membrane becomes at first serrate, and then undulate, thickened and crumpled. Finally, the fully formed inclusion body, which is deeply acidophilic, homogeneous, and well circumscribed with a sharply defined margin, lies in the center of the nucleus surrounded by a clear halo that separates almost its entire circumference from the thickened, undulate, nuclear membrane (Fig. 6).

The second variety of changes in the nuclei is almost as common as the developmental sequence which has been described above. In a few respects the two processes are similar and it is impossible to determine whether certain intranuclear bodies are a part of the first or the second theoretical sequence of changes.

In the following description an attempt has been made to reconstruct the steps in the development of the second type of intranuclear bodies. The well preserved periportal parenchymatous cells contain a few examples of the early stages. As one progresses toward the less viable central zones a great many nuclei are affected and the more

advanced stages become the most interesting feature of the histology. The earliest stage seems to be preceded by an increase in the size of the nucleus. The strands of chromatin become interrupted, chromatin material loses its affinity for basic dyes and the chromatin as well as the nucleolus seems to disappear as if by lysis. Tiny round, and often sharply defined, regularly spaced, pale blue granules appear and in almost every instance seem to fill the entire nucleus (Fig. 7). Rarely there are persistent remnants of chromatin and nucleoli which are margined along the nuclear membrane. These delicate granules, which can be resolved definitely into distinct unit structures, occasionally are amphophilic or lightly acidophilic, but even when acidophilic their uniform punctate appearance tends to segregate them from the small, irregular pink granules which seem to form the elementary units of the inclusion bodies of the first type. Nevertheless, there are certain nuclei in which the elementary bodies of the second type are surrounded by a clear zone which partially separates them from the nuclear membrane (Fig. 8). In these nuclei one can imagine a series of gradations through which a typical inclusion body of the first type might have been formed. However, this is not apparent. There is a tendency for these small round granules to maintain their basophilic or lightly acidophilic nature and to fade into a structureless homogeneous nucleoplasm which varies from pale pink to dark blue and almost invariably fills the entire nucleus (Figs. 9 and 10). As one approaches the zone of necrosis gradual dissolution of parenchymatous cells supervenes and the homogeneous nuclei become dark blue to purplish red (Fig. 11). The nuclear membrane, at first delicate and distended, in the progressive stages becomes thickened and irregular or serrate. Finally it seems to fuse with the intranuclear plasm as the liver cell shows evidence of disintegration (Fig. 12). The cytoplasm of the liver cells that exhibit this peculiar general type of intranuclear morphology is usually pale, delicately granular, slightly vacuolated and swollen in the early stages. The cytoplasm in the later stages becomes less granular, more homogeneous and more intensely basophilic or acidophilic. In the marginal areas of necrosis where deeply basophilic and acidophilic nuclei are abundant the cytoplasmic membranes are no longer detectable and groups of liver cells seem to have flowed together to form irregular cytoplasmic masses containing several closely approximated, circular or elliptical, basophilic or

lightly acidophilic bodies, which represent the remains of the nuclei and their homogeneous content (Fig. 12). Eventually, there is complete dissolution, often preceded by a loss of differential staining reactions.

The various special stains are of no significant value, but it seems worthwhile to record briefly the staining reactions of the intranuclear bodies in tissues fixed in Zenker's fluid. In general, all granules and the fully formed acidophilic inclusions are colored pale red by Mallory's anilin blue-acid fuchsin-orange G collagen stain. The homogeneous variety of the second sequence of nuclear change is pale red to dark maroon (Figs. 10 and 11). After hematoxylin and eosin the Gram-Weigert method for the demonstration of bacteria stains the typical large intranuclear inclusions dark red or maroon (Figs. 5 and 6). The punctate granules, as described in the second series of changes, are pale purple (Figs. 7, 8 and 9), while those filled with the homogeneous substance are magenta (Fig. 11). Mallory's phosphotungstic acid hematoxylin stains the abnormal intranuclear granules a pale purple. Not infrequently they exhibit a slight orange tinge. The typical intranuclear inclusions are dark purple (Figs. 5 and 6). The homogeneous nuclei vary from pale purple to almost black (Figs. 10 and 11). The inclusions do not retain the Ziehl-Neelson carbol fuchsin stain for acid-fast organisms. The sections stained with hematoxylin-eosin and eosin-methylene blue are comparable to those which are stained with Giemsa. The intensity and sharpness of detail, as obtained by Wolbach's modification of the Giemsa stain, make these sections most satisfactory for study.

An attempt was made to demonstrate bacteria. The Ziehl-Neelson carbol fuchsin stain discloses no acid-fast organisms. The tissues which were treated by Levaditi's method for impregnation of spirochetes contain no demonstrable treponema. The Giemsa and eosin-methylene blue stains disclose rare clumps of bacilli in the liver and adrenals. They are Gram-positive, and are not situated specifically in areas of necrosis. Similar organisms unaccompanied by necrosis are found in the lungs, spleen and vessels of the wall of the ileum.

Adrenals: In the peripheral subcapsular portions of the cortex of each adrenal gland there are numerous small focal areas of necrosis. The fundamental changes in these areas are similar to those in the broad fields of degeneration in the liver. The lesions are so small that many can be included in a high dry microscopic field. All ne-

crosses are acute. The primary histological changes suggest an autolytic type of parenchymal degeneration followed by disruption of sinusoids and consequent extravasation of red blood cells. In the more advanced lesions an agglomeration of the necrotic parenchymal elements and blood cells into structureless masses is characteristic. No significant inflammatory reaction is present.

One's attention, here, as well as in the liver, is attracted by the peculiar morphological alterations in the parenchymal cells. The great advantage in the study of the small early lesions is that the first stages of cellular degeneration and the progressive changes in the nuclei and cytoplasm are more clearly defined than in the liver. Intranuclear bodies of varied character which are identical with those in the liver cells are always present in the lesions. The morphological variations in the nuclear chromatin seem to precede or accompany the development of the intranuclear structures. The changes in the cytoplasm of most of the cells appear to follow the changes in the nuclei, because many cells, especially at the periphery of the lesions, have prominent intranuclear bodies without detectable abnormalities of the cytoplasm. The earliest evidence of cytoplasmic change consists of swelling, diminution in affinity for acid stains, reticulation and vacuolation. The cytoplasmic membrane is distended and the cell tends to be circular in outline. This is not accompanied by any apparent local increase in vascularity or significant disturbance of the general structural relations of the various cortical elements. In the more advanced lesions the cytoplasm is disintegrated and the cytoplasmic membrane often is disrupted. The nuclei and intranuclear bodies are almost indistinguishable. The vascularity is increased. The most severe lesions are characterized in their central portions by a fusion of the necrotic parenchymal elements and extravasated blood cells into structureless acidophilic masses. Peripherally, the progressive stages of cellular degeneration are found. In all instances the almost complete absence of leukocytic infiltration in the involved areas and the scant evidence of crystallized fibrin are consistent and inexplicable findings.

DISCUSSION

It is not within the scope of this presentation or within the range of the writer's experience to enter at great length into the spirited

polemics that have characterized the dissertations of morphologists and bacteriologists concerning the nature and significance of "inclusion bodies." We know that there are certain viruses which have many of the properties of living matter, that these agents are ultravisible in size, that they may pass through the pores of filters which withhold ordinary bacteria, that they are capable of producing disease and that the pathology of the disease is characterized by the presence, singly or in combination, of intranuclear or intracytoplasmic masses which are called "inclusion bodies." The similarity of structure and mode of formation of inclusion bodies in different filtrable virus diseases often make it difficult to distinguish the type of virus disease by a microscopic study of the inclusion bodies. Nevertheless, there frequently are certain histological differences which may enable one to classify the virus on the basis of the morphology of the inclusions which are associated with it. These differences need not be considered fully in this presentation.

The morphology of the intranuclear bodies in the present case will allow, within the limits of our knowledge, but one conclusion. Here we must be dealing with a disease which was produced by a hitherto unknown virus, filtrable in nature and of small physical dimensions, or by a known virus which has selected unusual sites for localization and which has exhibited its pathogenicity in an unique manner. There is no justification for assuming that the unit structures, which at times could be resolved as tiny granules, were actual single microorganisms. Neither is there any justification for assuming that they are not the virus bodies or clusters of those bodies, which must have been instrumental in the production of the extensive hepatic and adrenal necrosis. In this regard it may be said that certain rickettsiae, such as the *Dermacentroxenus rickettsii* of spotted fever, have been accepted as pathogenic microorganisms. These may inhabit the nuclei of cells in ticks (Wolbach¹). They have been cultivated in tissue cultures in the nuclei of infected mammalian mesenchymal cells (Pinkerton and Hass²). These microorganisms are often no larger than the elementary bodies, especially those of basophilic nature, in the nuclei of the parenchymatous cells of the liver and adrenal of the present case. The same may be said in regard to the elementary bodies of many other virus diseases. In Zenker-fixed tissues there is an undeniable resemblance between the intranuclear microorganisms of spotted fever and the intranuclear structures in

Figure 7. Not only is this true but it is quite apparent that the inclusion bodies which are formed by intranuclear masses of spotted fever rickettsiae, as demonstrated in tissue cultures, are similar to certain intranuclear inclusion bodies in various filtrable virus diseases.

It has been contended by certain authors that the acidophilic nature of inclusion bodies in general militates absolutely against the belief that they are composed of microorganisms. This does not seem entirely valid because in the present instance, as well as in herpes and experimental spotted fever, the inclusions and their constituent parts may be basophilic, amphophilic or acidophilic. Neither can such a simple criterion as the staining reaction be depended upon to indicate whether or not the virus inhabits the sphere of the inclusion body. It seems that the restrictions of microscopic vision will not allow the student to transgress the barrier, which arbitrarily has been thrown up between visible viruses which are recognized as intracellular inhabitants, and the ultraviolet viruses which are characterized by the presence of intracellular inclusion bodies. A few workers, notably Goodpasture,³ have produced evidence that the ultraviolet filtrable virus may be intimately associated with the inclusion body. A further important bond of similarity is that the continued cultivation of so-called filtrable viruses, as well as the rickettsiae, depends upon the presence of living cells in the medium. It is difficult to draw hard and fast lines between the two classes of pathogenic agents, one ultraviolet and characterized by inclusion bodies and the other visible and characterized by intercellular masses of microorganisms similar to inclusion bodies.

Let us compare briefly the present disease with those virus diseases that give rise to intranuclear inclusion bodies in the liver of man or animal, and with those instances in which inclusion bodies have been found in the liver independent of any established cause.

Yellow fever is a disease which presumably is caused by a filtrable virus that gives rise to necrosis of the liver and intranuclear inclusion bodies in the parenchymal cells. The inclusion bodies are very infrequent in human cases but are commonly found in the livers of monkeys in which the disease has been produced experimentally (Klotz and Belt,⁴ and Cowdry and Kitchen⁵). A comparison of the inclusions of the present case with those of experimental yellow fever in monkeys revealed superficial resemblances between a few

selected inclusion bodies. On the whole the morphological changes were unlike those of yellow fever.

Intranuclear and intracytoplasmic inclusion bodies have been noted from time to time in the liver, lungs, pancreas, thyroid, adrenals, kidneys and salivary glands of infants. A group of 25 cases was reported by Farber and Wolbach.⁶ The inclusions in these cases apparently were identical with those which various authors, especially Goodpasture and Talbot⁷ and VonGlahn and Pappenheimer,⁸ have reported and collected from the literature. A comparison of the present case with the material studied by Farber and Wolbach, and with the descriptions of the collected cases, yielded no similarities which would confuse the "protozoan-like" cells and their inclusions with the inclusion bodies of the present case. Neither has any pathogenic importance been attached to the "protozoan-like" cells with intranuclear inclusions, while it seemed reasonable to believe that the injury to the liver and adrenals of the case under discussion was due to specific localization of a virus.

McCordock and Smith⁹ listed a series of infants in which there were intranuclear inclusions. Case 3 of Group 1 had foci of necrosis in the liver and suprarenal glands. There was no detailed description of the histology. It is possible that their case may have much in common with the malady described in this report.

VonGlahn and Pappenheimer⁸ described intranuclear inclusion bodies in the intestine, liver and lungs of an adult who had a hepatic abscess and ulcerations of the cecum. They stated that the inclusions were identical with those that have been described in the viscera of infants. Dr. William VonGlahn has permitted the writer to study a section of the liver of their case. Neither the structure of the inclusion bodies nor the large "protozoan-like" cells which contained them was similar to the findings in the present case.

Rift Valley fever is a non-fatal virus disease which is characterized by focal necrosis in the liver of certain susceptible animals, such as sheep, goats, rats, squirrels, voles and wood mice. The inclusion bodies are restricted to the nuclei of the parenchymatous cells of the liver. The intranuclear inclusions are similar to those of yellow fever (Findlay¹⁰). The writer is indebted to Dr. G. M. Findlay for a section of the liver of a mouse with Rift Valley fever. A comparison with the liver of the present case disclosed similarities between many inclusion bodies. However, the resemblances were not sufficient to

admit the acceptance of close relationship or identity of the two processes.

Pacheco's parrot virus gives rise to characteristic intranuclear inclusion bodies, which may be found in the liver and other organs of parrots and parrakeets (Pacheco and Bier,¹¹ Rivers and Schwentker¹²). The inclusion bodies are not unlike those that have been found in yellow fever and Rift Valley fever. Although hepatic necrosis occurs in the susceptible avians, the pathogenicity of this virus for humans has not been demonstrated. The author is indebted to Dr. Thomas Rivers for a section of the liver of a parrakeet which died of this disease. The similarity between a few of the inclusions in the present case and those of the parrakeet disease did not distract from the great dissimilarity of the majority of the inclusions.

Findlay¹³ described intranuclear bodies in a strain of Clacton mice. These bodies, which in many respects resembled hypertrophied nucleoli, appeared in the liver cells of Banbury mice that had been inoculated with a suspension of liver tissue of Clacton mice. Evidence was brought forward to suggest that the intranuclear bodies were caused by a filtrable virus of low pathogenicity.

Cowdry and Scott¹⁴ described intranuclear inclusion bodies in the livers of dogs. Covell¹⁵ found intranuclear inclusions in livers of monkeys. In each instance no ultramicroscopic virus was demonstrated.

The possibility of a localization of the herpes virus in the liver and adrenals of the present case must be considered seriously. The inclusion bodies in many respects were similar to those that arise in herpetic (herpes simplex) infections. The nature of the development of the inclusions, their morphology, their staining reactions, the presence of basophilic granules, the concurrence of basophilic and acidophilic material in several inclusions, the margination of chromatin and nucleoli, the "halo" around many of the typical well developed bodies, and other features which have been given in detail in the microscopic description, revealed an undeniable resemblance to and frequent identity with the morphology of the inclusion bodies of herpes. By no means, because of the similarity of intranuclear inclusion bodies in many diverse virus diseases, could one state that the present case illustrated an instance of herpetic infection of the liver and adrenals. However, if one were forced to select the etiological agent from the group of well established filtrable viruses, the

herpetic virus would be favored as the most probable causative factor in this singular disease. Goodpasture and Teague¹⁶ were able to demonstrate intranuclear inclusion bodies in the parenchymatous cells of the liver and adrenals of rabbits in those areas where they had injected the herpes virus. Cowdry and Kitchen⁵ obtained the same results by injection of the herpes virus into the livers of monkeys.

The portal of entry and the route of infection in the present case were not determined. It was possible that the umbilical cord may have served as the site of the primary infection, although no local lesion was demonstrated. It seemed possible that the virus may have been introduced into the infant by transfusion with the father's blood. The peculiar persistence of viruses in the tissues and fluids of humans and animals long after the disease process has subsided is well recognized. Therefore, the apparent healthy condition of the father would not militate strongly against the transmission of a virus by transfusion. There was another possibility which may be considered in the light of our knowledge of virus III infections of rabbits. This latent agent apparently lies dormant in the testes of normal rabbits. It attains virulence and produces disease only after repeated passage through the testes of rabbits. It is possible, but very unlikely, that a similar dormant species virus may have inhabited the blood of the father. As has been stated in the clinical record, the infant failed rapidly after the transfusion and died 12 hours later. This deserves repetition and emphasis only because the clue to this disease remains obscure. A careful study and analysis of similar cases may afford some inquisitive person the opportunity to transmit the disease to animals and identify the pathogenic agent. For the present the description and interpretation of the pathology of this singular malady must suffice.

SUMMARY AND CONCLUSIONS

1. A case of a 7 months premature infant, who was afflicted with a fatal disease characterized by hepato-adrenal necrosis and intranuclear inclusion bodies in the parenchymatous cells of the liver and adrenal cortex, is described.
2. It is assumed that the unique lesions must have been produced by a filtrable virus.
3. No similar case has been found in the literature.

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DESCRIPTION OF PLATES

PLATE 15

FIGS. 1-12. Photomicrographs of representative cell nuclei of the hepatic parenchyma. These contain "inclusion bodies" of various types, as described in detail in the microscopic descriptions. Similar intranuclear "inclusion bodies" were found in the focal necroses of the adrenal cortex. $\times 2800$.



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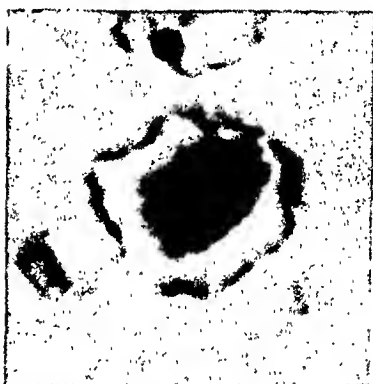
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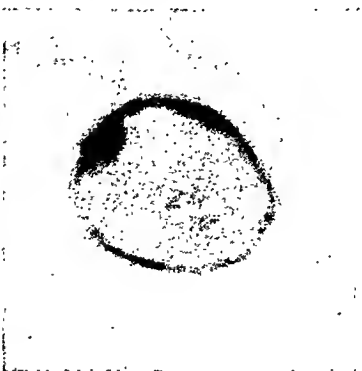
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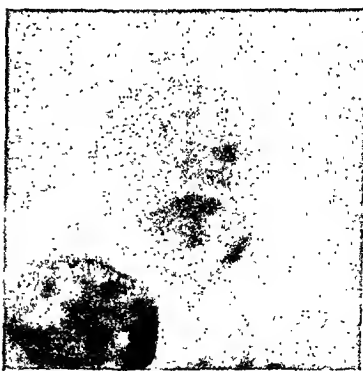
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12

PLATE 16

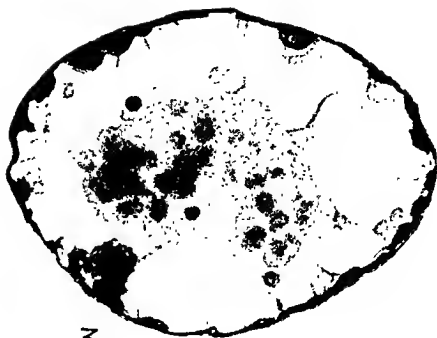
FIGS. 1-12. Camera lucida drawings of nuclei of hepatic parenchymal cells. The "inclusion bodies" which are contained in these nuclei are of the same type as those in Plate 15. Corresponding nuclei have the same numbers in the two plates (Figs. 1-12). $\times 3200$.



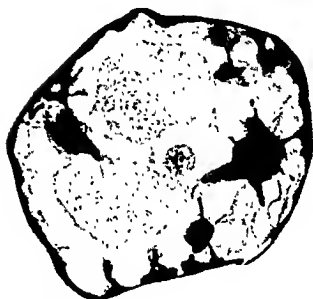
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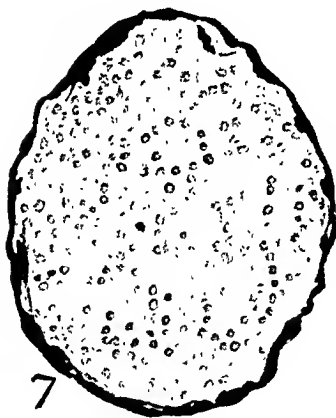
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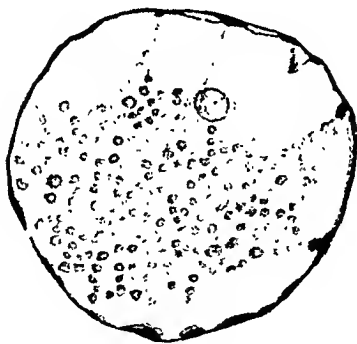
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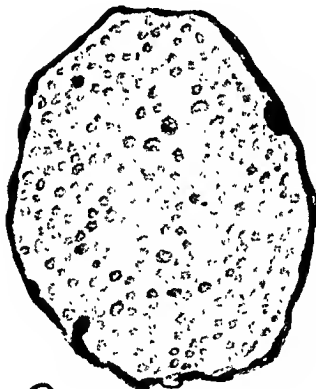
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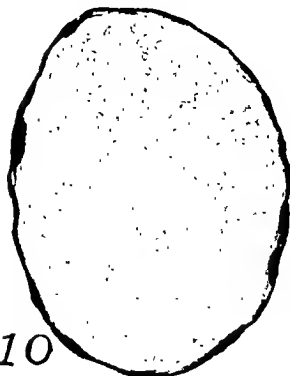
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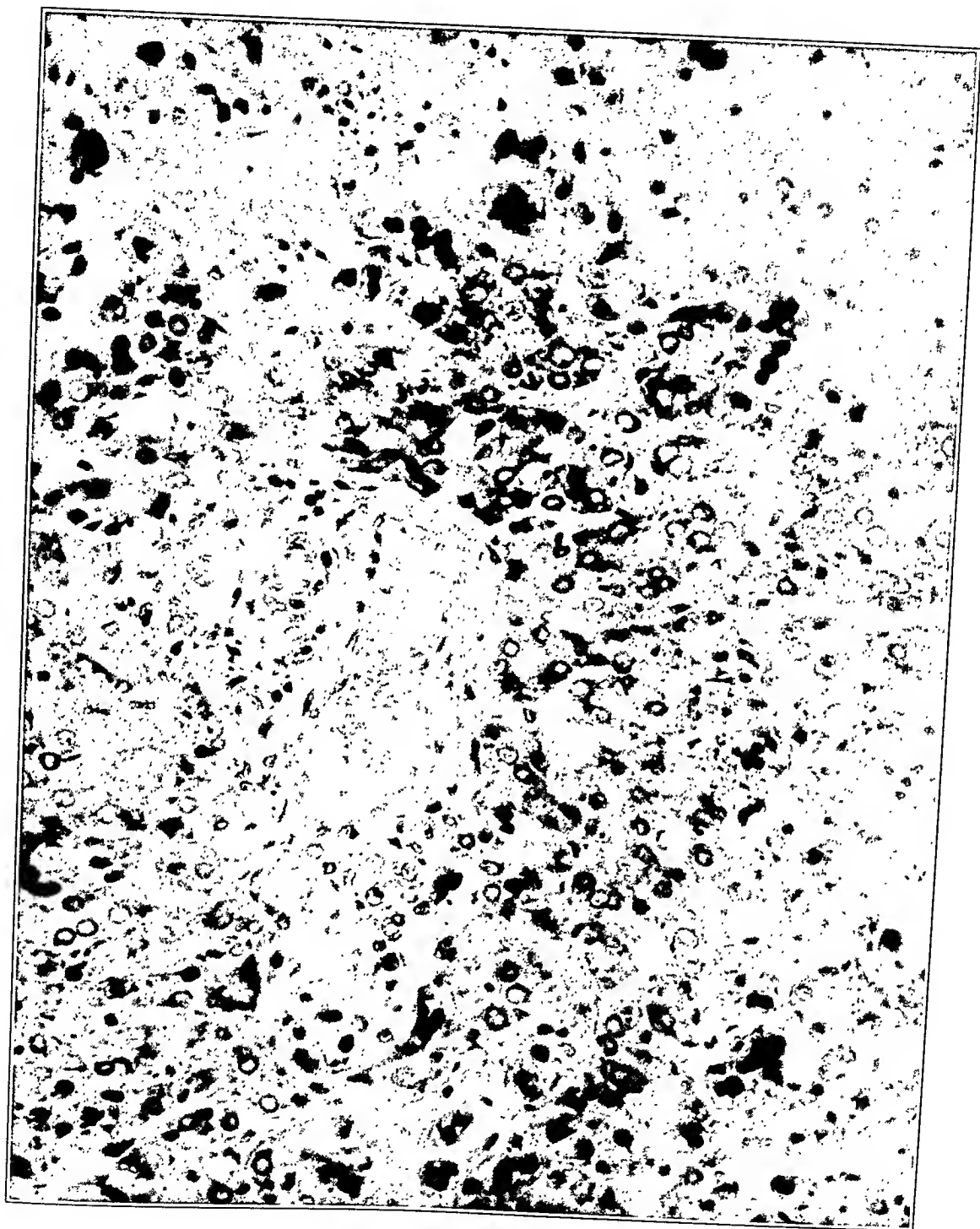


12

E. P. Hoff

PLATE 17

FIG. 13. A photomicrograph of a representative periportal area such as was considered fully in the description of the histopathology of the liver. $\times 150$.



13

MYOCARDIAL LESIONS IN SUBACUTE BACTERIAL ENDOCARDITIS *

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In a previous communication ¹ rheumatic changes in the myocardium of children dying from subacute bacterial endocarditis were reported. Because of the fact that various other changes in the myocardium were observed during this study it seemed of interest to examine the hearts of a large group of patients, both adults and children, dying from subacute bacterial endocarditis, with special reference to myocardial changes. This seemed the more worth while in view of the discrepancy of opinions as to myocardial changes in subacute bacterial endocarditis. While Blumer ² and others stated that the myocardium in subacute bacterial endocarditis is only rarely involved Clawson, ^{3,4} Libman, ^{5,6,7} and others emphasized the frequent occurrence of myocardial lesions.

In the following, a short abstract of the more important pertinent literature is given and the results of a study of the myocardium of thirty-five hearts of patients dying from subacute bacterial endocarditis are reported.

LITERATURE

Murray ⁸ in 1922 noted that early in an attack of subacute bacterial endocarditis the heart muscle shows little response to the infective processes and the patient shows no evidence of cardiac failure.

Blumer ² in 1923 stated that the relatively slight involvement of the myocardium is a special peculiarity of subacute bacterial endocarditis. In 150 autopsies he reported, conditions of the myocardium were mentioned only thirty times. In twelve instances the recorded lesion was an evidence of toxemia, such as generalized fatty degeneration or cloudy swelling. Chronic interstitial myocarditis was noted eight times, acute myocarditis twice, small abscesses in the heart muscle three times, focal necrosis twice and infarcts twice.

* Aided by a grant from the Nelson Morris Foundation.
Received for publication July 30, 1934.

Starling ⁹ in 1923 observed that in subacute bacterial endocarditis emboli are frequently found, but that the myocardium is rarely involved, thereby differing from rheumatic endocarditis.

Libman ⁵ in 1923 reported that whenever lesions are found in the heart muscle in acute bacterial endocarditis they consist in the main of polymorphonuclear leukocytic infiltrations. In cases of subacute bacterial endocarditis, however, one finds an essentially round cell interstitial lesion, which is not present in all cases and is not specific.

Clawson ³ in 1924 analyzed 220 cases of endocarditis. The myocardium of fifty-four hearts with subacute bacterial endocarditis revealed definite indications of inflammation in thirteen instances. The character of the exudate was mononuclear in all but two in which polymorphonuclear leukocytes were found. While cardiac symptoms might not be common early in the course of the disease, at death evidences of cardiac failure are conspicuous findings. In this series evidence of cardiac failure, as manifested by edema in its various forms and passive hyperemia of the liver, was common.

Murphy and Dugan ¹⁰ in 1924 stated that the myocardium beneath the vegetations shows a rather extensive infiltration by polymorphonuclear leukocytes and round cells.

Libman ⁷ in 1925 reported that among twenty-seven patients with subacute bacterial endocarditis in the "bacterial-free stage" myocardial insufficiency was a determining factor in the causation of death of fourteen. Five patients revealed myocardial insufficiency alone. He also stated that "the part played by myocardial insufficiency is striking."

Bierring ¹¹ in 1926 noted that "cardiac symptoms except for their fundamental significance are among the least prominent of all."

Thayer ¹² in 1926 stated that while in acute rheumatic endocarditis the myocardial lesions are prominent, in bacterial endocarditis they are generally subsidiary. Occasionally focal areas of interstitial infiltrations with round cells or leukocytes are found in the myocardium in subacute bacterial endocarditis.

Rothschild, Sacks and Libman ¹³ in 1927 reported that the examination of the myocardium in subacute bacterial endocarditis discloses, in the majority of cases, focal lesions consisting of cellular infiltrations. Although these changes are seen mainly in the ventricular musculature, the infrequency of changes in the ventricular form of the electrocardiogram necessitates the assumption that they

are generally without significant effect on the intraventricular conduction.

Clawson ⁴ in 1928 stated that the greater frequency of abscesses in subacute bacterial endocarditis than in other forms of endocarditis evidently results from lodging of infected emboli from the valves in the myocardium. He also stated that myocarditis is even more frequent in subacute bacterial endocarditis than in acute or recurrent rheumatic endocarditis.

Hamman and Rich ¹⁴ in 1933 reported 2 cases of subacute bacterial endocarditis. In the first case the myocardium was essentially normal. The second revealed focal areas where the myocardium had been replaced by scars. There also were many minute infarcts and an occlusion of a small branch of the coronary artery by an organized thrombus. Also, fragments of infected vegetations were found in branches of the coronary arteries.

Longcope ¹⁵ in 1933 stated that some form of pathological process may be found in the heart muscle of about one-half the cases of bacterial endocarditis that come to autopsy.

METHODS

Thirty-five hearts were examined. Sections from various portions of both ventricles were embedded in paraffin and stained with hematoxylin-eosin. The Gram-Weigert and the Van Gieson stains were also used. Frozen sections were often cut and stained with Sudan III to demonstrate the presence of fat. In some instances serial sections were cut from a whole block and stained with hematoxylin-eosin. The Prussian blue reaction was employed to determine the presence of iron-containing pigment.

RESULTS

Grossly the myocardium almost invariably was softer than normal, its cut surface of a boiled appearance and the architecture obscured. Just beneath the endocardium many minute yellowish streaks were often observed, which occasionally were arranged in the form of tiger stripes. These were particularly evident in the papillary muscles of both ventricles. In some hearts the myocardium was traversed by grayish yellow and grayish red streaks, and had a peculiar speckled appearance. Occasionally, circumscribed, minute yellow nodules, or larger, soft yellow areas were encountered which were surrounded by hemorrhagic zones.

Only in 1 case could an embolus be demonstrated grossly in the coronary artery. The aortic valve in this heart was almost completely destroyed and all three cusps were practically replaced by large, soft, grayish red vegetations. Mycotic aneurysms were found in the sinus of Valsalva corresponding to the left and posterior aortic cusps. The distal portion of the circumflex branch of the coronary artery at the point of origin of the ramus marginis obtusi was occluded by an embolus which was reddish gray, soft, and similar in every respect to the vegetations on the aortic valve.

In six hearts petechial hemorrhages were encountered. More commonly the subendocardial layer was involved but occasionally the myocardium in an area at a distance from the endocardium was affected.

HISTOLOGICAL EXAMINATION

Table I summarizes the histological findings of these thirty-five hearts.

Degenerative changes were present in all cases. The muscle fibers were swollen, their striations in some fields could not be made out at all and the sarcoplasm was distinctly granular. In many instances numerous, minute fat globules were found distributed throughout the sarcoplasm. In addition to these changes the following abnormalities were noted.

Petechial Hemorrhages: In 6 cases petechial hemorrhages in the myocardium were found microscopically. The hemorrhages were apparently very recent and the red blood corpuscles surrounded minute vessels. Neither emboli nor white cells were found in these vessels.

Acute Inflammatory Changes, Foci of Necrosis and Abscesses: Acute inflammatory changes were found in the myocardium in 15 cases. Accumulations of polymorphonuclear leukocytes were usually present in the perivascular spaces and often extended into the interstitial tissue between the heart muscle fibers. Only occasionally a few lymphocytes were seen. In 10 cases the heart muscle fibers themselves were involved. Small foci of necrosis of portions of individual muscle fibers were easily observed. The necrotic centers were often infiltrated and surrounded by polymorphonuclear leukocytes and an occasional lymphocyte and endothelial cell. In several instances clumps of bacteria were found within the necrotic

TABLE I

No. of case	Pete- chial hemorrhages	Acute myocarditis	Foci of necrosis and abscesses	Minute infarcts			Emboli	Perivascular, subacute, and chronic inflammation	Aschoff bodies	Perivascular fibrosis	Remarks
				Recent	Organized	Healed					
1		+	+	+	+		+				
2		+	+								
3	+				+				+	+	
4		+	+		+		+	+	+		Portion of vege- tation form- ing embolus
5		+	+		+		+		+		
6					+	+		+	+		
7		+	+		+		+		+	+	
8					+			+	+	+	
9		+			+	+	+			+	
10				+	+	+					
11					+	+		+			
12					+	+			+	+	
13		+	+		+		+				
14	+	+	+		+		+			+	
15								+		+	
16					+	+	+				
17	+		+								
18					+						
19		+			+	+	+				
20		+	+						+	+	
21	+	+	+		+	+	+		+		Abscess in bundle of His
22					+			+			
23					+		+			+	
24		+	+				+				
25					+		+	+		+	

TABLE I (Continued)

No. of case	Pete- chial hemorrhages	Acute myocarditis	Foci of necrosis and abscesses	Minute infarcts			Emboli	Perivascular, subacute, and chronic inflammation	Aschoff bodies	Perivascular fibrosis	Remarks
				Recent	Organized	Healed					
26							+	+	+	+	
27			+		+	+			+	+	
28					+	+			+	+	
29					+	+	+	+			
30					+		+	+		+	
31		+	+						+		
32					+	+	+		+		
33					+			+			
34	+	+	+		+		+			+	
35	+	+	+		+	+					

center. In 5 cases minute vessels were filled with bacteria which in turn were surrounded by polymorphonuclear leukocytes. Sometimes bacteria were seen in the peripheral portions of the lumens of the large arteries. Special stains revealed that the bacteria were invariably Gram-positive cocci arranged in small groups.

The acute interstitial changes and the minute parenchymal abscesses were almost always found in the same heart. In only 2 cases were acute interstitial changes alone noted. The common location of the abscesses were areas just beneath the pericardium and the endocardium of the left ventricle. Only occasionally was it necessary to examine many sections before these lesions were encountered.

Minute Infarcts: They were most frequently encountered. In general, three stages could be differentiated. The first, earliest stage, was only seen twice. It was characterized by areas of necrosis revealing no details of heart muscle fibers. These areas were more or less homogeneous, eosinophilic and were surrounded by clumps of cocci, polymorphonuclear leukocytes and a few red blood corpuscles. These infarcts varied, but only occasionally did they attain a conspicuous size.

The second stage was commonly encountered (in this series, twenty-eight times). This was the stage of organizing infarcts. Many spindle-shaped cells were arranged in parallel rows replacing the heart muscle fibers. Very occasionally, lymphocytes were seen scattered among these cells. Phagocytic cells were often encountered, their cytoplasm filled with a reddish granular pigment which gave a positive iron reaction. There was also a scant new formation of connective tissue fibers and several small sized blood vessels extended through this region. In many sections which showed these infarcts, emboli were encountered in their vicinity. When emboli were not seen in the section revealing organization tissue they were, as a rule, found in an adjacent section. In one instance only, a large branch of the coronary artery contained, in addition to bacteria, some necrotic and calcareous material. This material was obviously a portion of a broken-off vegetation. Surrounding this vessel many polymorphonuclear leukocytes were seen, in addition to a number of foreign body giant cells.

The third stage was characterized by the formation of dense scar tissue. Only a few nuclei were noted in this old connective tissue. A few clumps of pigment granules and an occasional phagocytic cell filled with blood pigment led to the inference that these scars represented the healed stage of small infarcts. These scars replaced heart muscle fibers and were not confined to perivascular spaces.

Perivascular Cellular Infiltrations: (Non-specific Subacute and Chronic Inflammatory Lesions): In eleven hearts inflammatory lesions were encountered which were strictly confined to the perivascular spaces. The predominating type of cell was the lymphocyte. Only occasionally a few plasma cells or endothelial cells could be found among the lymphocytes. These cellular infiltrations did not extend into the adjacent parenchyma. They were present eleven times. In only 2 cases were these lesions encountered in the same hearts which showed acute inflammatory changes. In five hearts lymphocytes were found scattered in the perivascular spaces, in addition to a new formation of fibrous tissue which still revealed fibroblastic cells. In some instances transitions between cellular infiltrations and perivascular fibrosis could be demonstrated. Multinucleated cells, or other cells resembling those seen in the Aschoff body, were not observed in these fields. The cellular elements within the perivascular fibrous regions showed no specific arrangement.

Aschoff Bodies: In 14 cases Aschoff bodies were present in the myocardium. Ten of these cases were previously reported.¹ Again it should be emphasized that whenever there was doubt as to whether or not a lesion was an Aschoff body it was not diagnosed as such. Cellular infiltrations resembling Aschoff bodies were not included. The Aschoff bodies invariably consisted of infiltrations of large cells often showing a basophilic cytoplasm containing one, two or three nuclei, a few lymphocytes and an occasional plasma cell and polymorphonuclear leukocyte. These accumulations of cells were almost always found in the vicinity of the blood vessels. Occasionally, necrotic foci or a fibrin-like material were encountered in these areas. The large cells were seen in parallel rows, often assuming a typical palisade arrangement. The internal structure of the nuclei of some of these cells (Aschoff cells) could be compared with that of a spider web. Apparently, depending upon the pressure of the surrounding tissues, the cells were either compactly arranged, the Aschoff bodies presenting an elongated appearance, or the cells were well separated from one another, the Aschoff bodies appearing rather square or round.

Perivascular Fibrosis: A new formation of connective tissue in the perivascular spaces was frequently observed. Often no cellular elements could be found in these areas even in serial sections. In several instances a few lymphocytes or histiocytic cells were recognized. Occasionally the latter showed the characteristics of the Aschoff cell.

DISCUSSION

The petechial hemorrhages which were found in 6 cases were very recent. In these cases petechiae were also encountered in the skin or conjunctivae. It seems evident that petechiae in the myocardium have the same etiology as those found in the other locations, namely toxemia or bacteremia.

Fifteen out of thirty-five hearts revealed abscesses or foci of necrosis. It is probable that the foci of necrosis often were the precursors of the abscesses. *These abscesses undoubtedly are the result of small bacterial emboli lodging in minute vessels or capillaries causing first necrosis, and secondarily abscesses. Acute inflammatory changes without formation of abscesses were encountered twice, were confined to the interstitial tissue and justify the term*

acute interstitial myocarditis. Clawson stressed the greater frequency of abscesses in subacute bacterial endocarditis than in other forms of endocarditis. In his series abscesses were found in 21.5 per cent of the cases. Birch-Hirschfeld,¹⁶ as early as 1894, mentioned ulcerative endocarditis as one of the causes of purulent myocarditis. He also stated that foci of necrosis encountered in these cases may progress to abscess formation. Very close to the necrotic foci and often in the midst of the abscesses, capillaries filled with cocci were still recognizable. If larger vessels were involved, bacteria were recognized in the peripheral portions of the lumens. This indicates that these inflammatory processes, some with small areas of central necrosis, are not comparable to infarcts but are more likely the response to the toxic products of the bacterial emboli. Mönckeberg¹⁷ also stated that areas of necrosis did not correspond to the regions supplied by the affected vessels and that the necrotic foci therefore cannot be regarded as anemic infarcts.

The perivascular infiltrations consisting mainly of lymphocytes are noteworthy. These lesions are entirely different from those just mentioned. They also do not resemble Aschoff bodies. They are circumscribed subacute and chronic inflammatory lesions confined to the perivascular spaces and show no characteristics that give the impression of specific lesions.

The question arises as to whether or not these lesions can be regarded as Bracht-Wächter bodies. Bracht and Wächter¹⁸ described minute and larger areas of necrosis surrounded by lymphocytes and fibroblasts in the myocardium of a rabbit injected five times at 48 hour intervals with 2 or 3 cc. of broth cultures of *Diplostreptococcus rheumaticus*. In Rabbit 2, which was injected 4 times over a period of 14 days, the myocardium revealed irregularly distributed, more or less well defined, cellular infiltrations situated mainly in the interstitial tissue. Occasionally these foci extended into the muscle fibers themselves. A few necrotic muscle fibers were seen with calcification. Most of the cells were lymphocytes. Fibroblasts, and occasionally plasma cells, were also present. In Rabbit 3, which was injected four times over a period of 16 days, the myocardium revealed long, spindle-shaped, thread-like connective tissue nuclei and streaks of fibrosis in the midst of the muscle fibers.

From this description it is difficult to determine just what a Bracht-Wächter body is. This is particularly so because of the loca-

tion of the lesions described by these authors. The lesions in Rabbit 2 were found principally in the interstitial tissue, while the lesions in Rabbits 1 and 3 were found predominating within the parenchyma. If the lesions found in Rabbit 2 are regarded as so-called Bracht-Wächter bodies, then it must be considered that their description resembles the perivascular infiltrations seen in the myocardium in these cases of subacute bacterial endocarditis. It is interesting to note, however, that Rothschild, Sacks and Libman¹³ described Bracht-Wächter bodies as follows. "Examination of the myocardium in subacute bacterial endocarditis discloses in the majority of cases focal lesions consisting of cellular infiltrations (chiefly of round cells) in areas where the muscle fibers have undergone degeneration or necrosis. These are the so-called Bracht-Wächter lesions which differ from the Aschoff bodies in certain essential particulars. They are frequently inconspicuous in size and distribution, but at times they are widely diffused throughout the myocardium, and the individual lesions may assume considerable proportions."

Libman⁶ stated as follows. "In cases of subacute bacterial endocarditis there is often present a focalized lesion in the myocardium known as the Bracht and Wächter lesions. These are foci which consist mainly of lymphocytes and are found in the muscle fibers themselves — the Aschoff bodies being found outside the muscle fibers. The Bracht-Wächter bodies have been reproduced experimentally, but nobody has been able to reproduce the Aschoff bodies."

Bishop *et al.*,¹⁰ mentioned Bracht and Wächter bodies in cases of subacute bacterial endocarditis. They describe them as scattered areas of focal cellular accumulations consisting of large mononuclears, polymorphoneutrophiles and lymphocytes. The description of these authors, especially as far as location is concerned, corresponds much more to the lesions produced by Bracht and Wächter in Rabbit 3. This point will be discussed again later.

Because of the uncertainty of just what constitutes a Bracht-Wächter body, it seems wise to discard the term "Bracht-Wächter bodies" entirely and to use a rather descriptive term for lesions which by some investigators might be called Bracht-Wächter bodies. It may be mentioned in this connection that Mönckeberg,¹⁷ probably because of the three apparently different lesions described by Bracht and Wächter, stated that these authors were not able to pro-

duce any characteristic lesions in their experiments with streptococci.

Organizing infarcts (granulation tissue) were encountered frequently. The question arises whether the described lesions are organizing infarcts or organization tissue, the result of a primary localized inflammation. The presence of blood pigment free in the tissue and within phagocytic cells is in favor of infarcts. The sparsity of inflammatory cells and the preponderance of the spindle-shaped cells also speak against a primary inflammation. Finally, the presence of emboli in the smaller branches of the coronary arteries in the vicinity of these lesions aids in determining the origin of the granulation tissue. The emboli apparently arise from broken-up vegetations. It must be emphasized, however, that often many sections have to be cut through an infarct to locate the embolus.

It is much more difficult to determine whether or not the larger fibrotic lesions which were found replacing the muscle fibers were old infarcts. Their size, the presence of an occasional phagocytic cell loaded with pigment and a few clumps of pigment free in the tissue were taken as possible evidence of old infarcts. Also, the simultaneous findings of the organizing infarcts, old fibrous scars and organized emboli are in favor of the fibrous lesions being the healed stage of infarcts. When fibrotic changes alone were found they were not designated as healed infarcts but were merely referred to in a purely descriptive manner.

The organizing infarcts seem to be the most characteristic changes in the myocardium in subacute bacterial endocarditis. Possibly some of the lesions found by Bracht and Wächter in Rabbit 3 may be interpreted as infarcts. These authors, in addition to the changes described before, also found necrotic lesions with polymorphonuclear leukocytes, fibroblasts and lymphocytes classified as possible infarcts. It is also possible that some of the lesions in the myocardium produced experimentally by Thalheimer and Rothschild²⁰ can be interpreted as infarcts. They described them as follows. "Later the lesions became more proliferative. Fibroblasts soon became prominent and developed rapidly into a fibrous stage. Later fibrous tissue was found surrounded by healthy muscle fibers containing a few leukocytes. Not all the fibrous areas were circumscribed. Many were diffuse, their fibers running parallel to those of the myocardium, apparently having replaced the latter. Some of the focalized

lesions had a close relationship to the small and medium sized blood vessels. Occasionally, hyaline thrombi were found." However, these authors stated that the hyaline thrombi did not occur regularly and appeared to have had no relation to the lesions.

All the hearts in this series were taken from children or young adults. Because the collaterals of the coronary arteries are not yet developed in the young, it is easily understood why emboli in small branches of the coronary arteries caused infarcts. It is also possible that the reason for the development of infarcts in these hearts lies in the simultaneous involvement of several small branches of one coronary artery. It may be of interest to point out that emboli in the coronary artery resulting from broken-down thrombi are very rare, while emboli arising from vegetation or bacterial emboli are commonly encountered histologically in these vessels in subacute bacterial endocarditis. On the other hand, an embolus was recognized grossly only once. The destruction of the aortic valve with the resulting insufficiency of this valve may have aided in the lodging of emboli in the coronary arteries.

Aschoff bodies were encountered fourteen times. In Clawson's⁴ series of 60 cases of subacute bacterial endocarditis Aschoff bodies were found twenty-seven times. The significance of the findings of Aschoff bodies in subacute bacterial endocarditis was the object of a previously reported study.¹ It may again be stressed that the finding of typical Aschoff bodies in the myocardium in subacute bacterial endocarditis may be taken as evidence against the assumption that subacute bacterial endocarditis is the immune response in a previously hypersensitive patient. It would be difficult to explain why a tissue should respond simultaneously in two ways, namely with a hypersensitive reaction, of which the Aschoff body is supposed to be an example, and with an immune reaction, *i. e.*, subacute bacterial endocarditis. The Aschoff bodies were undoubtedly recent, indicating that rheumatic infection was present at the time of development of subacute bacterial endocarditis. It may also be of interest to point out that Aschoff bodies and abscesses were occasionally encountered in a single section. It may be mentioned that Thayer¹² also found Aschoff bodies associated with abscesses in the myocardium in one heart.

Perivascular areas of fibrosis were encountered fifteen times. In 6 cases they were seen in the hearts which also contained Aschoff

bodies and in 3 other cases they were present in hearts which revealed perivascular areas of non-specific subacute and chronic inflammation, but no Aschoff bodies. Only twice a perivascular fibrosis was encountered in a heart which showed both lesions. The perivascular areas of fibrosis were present in four hearts showing neither Aschoff bodies nor perivascular areas of subacute and chronic inflammation. Possibly the perivascular areas of fibrosis may represent the healing stage of both these lesions.

SUMMARY AND CONCLUSIONS

Myocardial changes were encountered in 35 cases of subacute bacterial endocarditis. These changes may be summarized as cloudy swelling, fatty degeneration, petechial hemorrhages, acute myocarditis, foci of necrosis and abscesses, areas of perivascular acute and chronic (non-specific) inflammation, minute infarcts, emboli in branches of the coronary arteries, Aschoff bodies and perivascular fibrosis. Minute infarcts were the most commonly encountered and the most characteristic lesions. Bracht-Wächter bodies are discussed and the conclusion reached that this term signifying specific lesions should be discarded because of the uncertainty of just what constitutes a Bracht-Wächter body.

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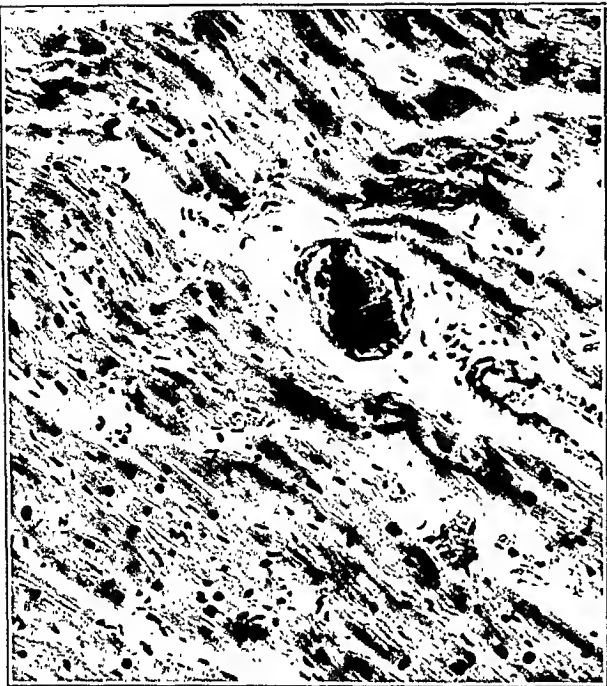
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DESCRIPTION OF PLATES

PLATE 18

- FIG. 1. Recent embolus in branch of coronary artery. Hematoxylin-eosin preparation. $\times 150$.
- FIG. 2. Bacterial embolus. Note the acute inflammatory changes in the wall of the vessel. Hematoxylin-eosin preparation. $\times 100$.
- FIG. 3. Organizing embolus. Hematoxylin-eosin preparation. $\times 175$.
- FIG. 4. Focus of necrosis. Note the moderate number of polymorphonuclear leukocytes. Hematoxylin-eosin preparation. $\times 200$.



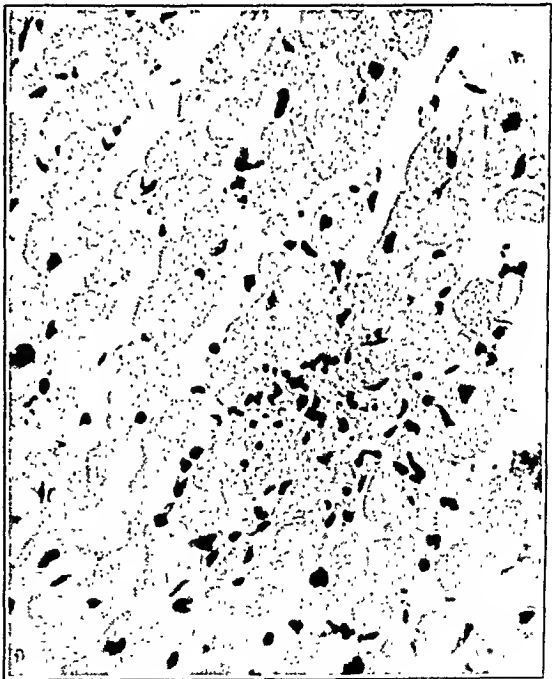
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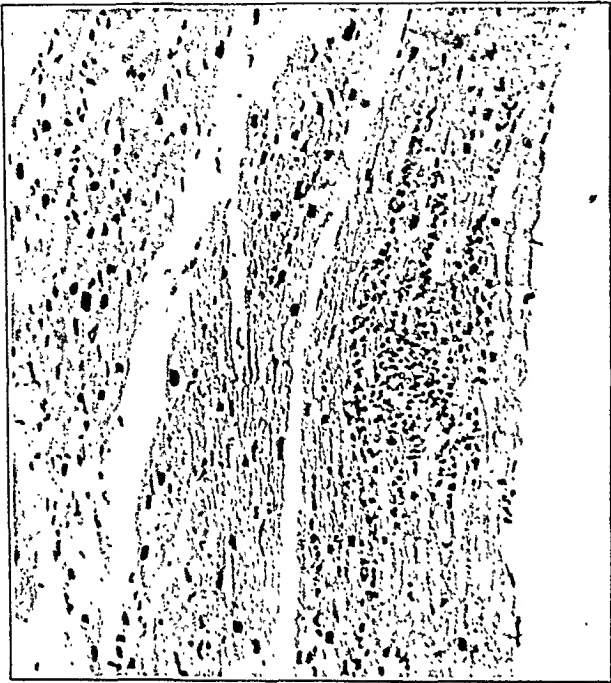
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PLATE 19

FIG. 5. Abscess in the bundle of His. Hematoxylin-eosin preparation $\times 125$.

FIG. 6. Abscess in the myocardium. Hematoxylin-eosin preparation. $\times 300$.

FIG. 7. Area of subacute and chronic (non-specific) perivascular inflammation.
Hematoxylin-eosin preparation. $\times 150$.



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6



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PLATE 20

FIG. 8. Organizing infarct. Note the large number of small vessels. Hematoxylin-eosin preparation. $\times 190$.

FIG. 9. Organizing infarct, higher magnification of Fig. 8. Note the pigment-containing cells. Hematoxylin-eosin preparation. $\times 400$.

FIG. 10. Healing infarct. Note the large number of fibroblasts and pigment-containing cells. Hematoxylin-eosin preparation. $\times 150$.

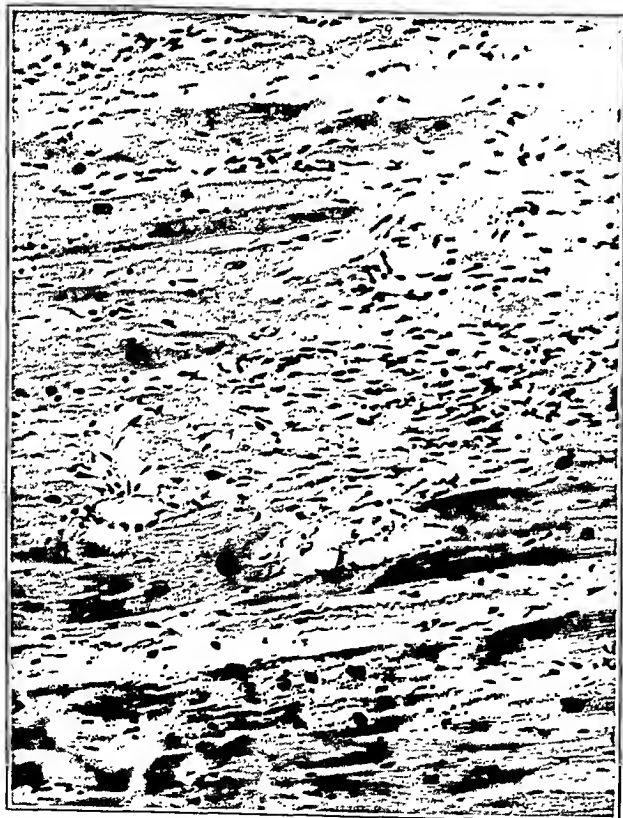
FIG. 11. Healing infarct, higher magnification of Fig. 10. Hematoxylin-eosin preparation. $\times 300$.



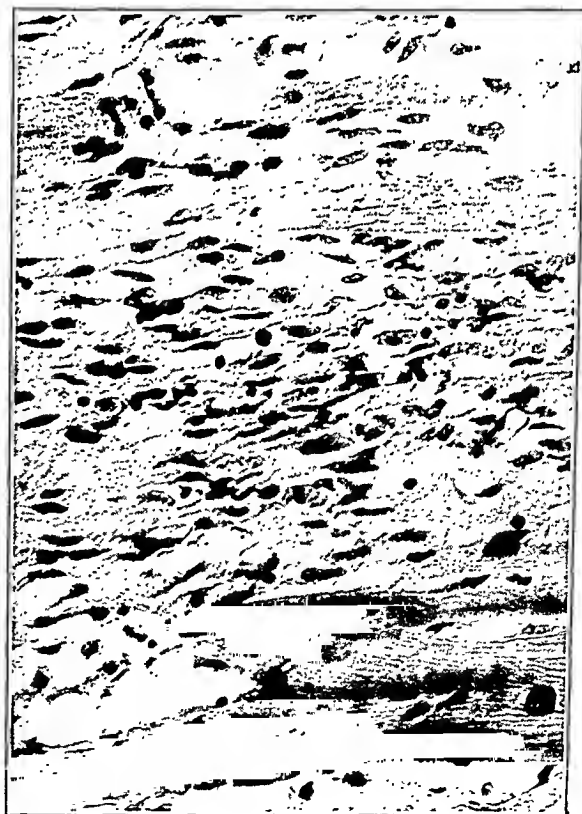
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HEPATIC INFARCTION *

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True infarction of the liver is rare, occurring even less frequently than an uncritical review of the literature would appear to indicate. Zimmerman, in 1930, and Cioni, in 1932, attempted to collect the reported cases of hepatic infarction, but careful evaluation of the data cited in the original reports reveals the inadequacy of the criteria upon which this diagnosis was made. Among the various lesions to which this designation has been erroneously applied are such conditions as "fatty infarcts," the so-called "atrophic red infarcts" of Zahn, localized areas of hemorrhage, marked passive congestion with hepatic cell atrophy, and small intralobular areas of focal necrosis occurring in biliary stasis. The fact that these lesions differ widely in their pathogenesis and morphological characteristics has led to a misconception of several important features of the process of true hepatic infarction.

According to the generally accepted definition, an infarct is an area of necrosis due to local anemia resulting from obstruction of circulation. In the case of organs with multiple vascular supply or with abundant collateral blood vessels, the development of this lesion is necessarily conditioned by several factors which are not operative in organs with a single circulation. The approach to this problem is particularly difficult in the case of the liver, since one must critically evaluate the relative importance, in this connection, of obstruction of each of the two afferent (hepatic artery and portal vein) and the efferent (hepatic vein) vessels, all of which possess numerous collaterals and anastomotic branches.

In arriving at the diagnosis of hepatic infarction, the final decision must be based primarily upon the gross and histological characteristics of the lesion itself which, in the main, are comparable to those of similar lesions in other organs. This criterion has been adopted by

* Received for publication August 3, 1934.

us in reviewing the cases reported in the literature, only 20 of which have been found acceptable on this basis. Seven additional cases are described which illustrate the red, pale, and organizing phases of this lesion, and one other is presented which we believe may represent the completely healed stage of hepatic infarction.

RED INFARCT

CASE 1. Jeff. Hosp. H-72. M. M., a white female, aged 32 years, receiving injection treatments for varicose veins, suddenly developed vascular thrombosis and inflammatory muscular lesions of the right leg on Dec. 24, 1933. Gangrene developed and the leg was amputated under ether anesthesia 4 days later. A systolic murmur, changing later to a double murmur, was heard over the apex of the heart, which was definitely enlarged. *Streptococcus hemolyticus* was cultured from the blood stream and from the amputation stump. The patient developed marked jaundice, widely scattered petechial hemorrhages and new areas of gangrenous involvement on the left hand and foot. Death occurred Jan. 9, 1934.

Autopsy Report

The autopsy was performed 12 hours postmortem. The anatomical diagnoses were acute vegetative endocarditis of the mitral leaflets and left auricle, embolic abscesses of the myocardium, embolic occlusion of the aorta at its bifurcation and of the intrahepatic portion of the left branch of the hepatic artery, thrombosis of the uterine vessels and of the larger branches of the pulmonary artery, and infarcts of the lungs, kidneys, spleen, brain and liver. The splenic and hepatic infarcts were contaminated by gas bacillus infection.

The liver, weighing 2170 gm., was soft, friable and greasy and presented a yellowish mottling which turned green on standing. In the lateral border of the left lobe there was a reddish brown area, 10 by 3.5 by 1.5 cm., sharply demarcated from the surrounding tissue by an uninterrupted, uniformly dark red, wavy line 2-3 mm. in width (Fig. 1). This line merged into a mottled zone of vascular congestion 1-2 cm. wide, which faded out gradually into the indistinct lobular markings of the remainder of the liver. The lesion extended about 1.5 cm. into the depth of the organ and the inner limits were sharper palpably than visibly. The cut surface was dry and dull, the lobular markings were effaced, thrombosed vessels were visible and the central portion was paler than the periphery, which was separated from Glisson's capsule by a dark red, homogeneous

zone 1-2 mm. wide. A large branch of the hepatic artery, extending directly into the area of infarction, was completely occluded by a gray thrombus originating 4.5 cm. proximal to the area of necrosis. The bile ducts and larger branches of the hepatic and portal veins were patent. The gall-bladder contained 20 cc. of thick, dark bile.

Microscopic Examination

In the microscopic sections distant from the infarct the hepatic cells are slightly pigmented, granular, vacuolated and necrotic in sporadic areas; the nuclei of other cells are frequently enlarged, hyperchromatic and multiple. In the Nile blue sulphate preparations blue and reddish granules and droplets are demonstrated in hepatic and Kupffer cells in scattered small areas. The lumens of the bile ducts contain myriads of bacteria and desquamated epithelial cells.

The infarct is regularly separated from the surface of the liver by a narrow subcapsular zone of compressed viable tissue showing evidence of hepatic cell regeneration. The branch of the hepatic artery leading to the area of infarction is completely occluded by an adherent loose network of fibrin, small masses of platelets and large numbers of leukocytes, red blood cells and masses of micrococci. The accompanying branch of the portal vein is patent. Within the area of infarction there is complete necrosis of all the cells but the arrangement and structural details are well preserved. The area is diffusely stained with hemoglobin and its derivatives which along with a small amount of lipoid material is contained within Kupffer and hepatic cells around the central and sublobular veins. The perisinusoidal tissue spaces are occasionally edematous and the sinusoids are dilated and empty as a rule, but sometimes contain the shadowy outlines of erythrocytes and hyaline thrombi which alternate with masses of granular and fibrillar debris, mycotic emboli or closely packed necrotic leukocytes. The reticular walls of the sinusoids are irregular in outline, often swollen and beaded, and sometimes split, frayed and torn. The necrotic hepatic cells are somewhat distorted and deranged, and most of their nuclei are invisible while others exist in outline form only, or as poorly defined, slightly basophilic structures embedded in dense homogeneous, deeply acidophilic cytoplasm stained diffusely with hemoglobin and containing granules

and needle-shaped crystals of pigment. The outlines of the canaliculi which persist are usually accentuated by the presence of fine granules of pigment in the bordering cytoplasm. The portal radicles are frequently reduced to smudges of acidophilic material and are distorted by the presence of large vacuoles containing cocci, large bacilli and acidophilic, granular material resembling precipitated albumin. The walls of the branches of the hepatic artery and portal and hepatic veins are all necrotic and their lumens either empty or partially filled with fibrinous or leukocytic thrombi, necrotic liver cells and unidentifiable débris.

This area of necrosis is sharply demarcated from the surrounding tissue by a wide, deeply red-staining zone in which all the sinusoids are distended with a dense, homogeneous, acidophilic material, practically devoid of erythrocytes. This abrupt change in the contents and staining reactions of the sinusoids with corresponding compression of liver cords and a marked increase in the hepatic cell pigmentation are the chief distinguishing characteristics between the two zones in which the morphology and staining properties of the hepatic cells are otherwise essentially similar. More externally, the sinusoids are usually dilated and packed with phagocytic cells and erythrocytes undergoing hemolysis.

PALE INFARCTS

CASE 2. Jeff. Hosp. H-167. E. T., a white female, aged 55 years, was suddenly seized on Dec. 15, 1933, with excruciating thoracic pain which radiated through to the back and down both arms, and which was associated with dyspnea, cyanosis, auricular fibrillation, low blood pressure and slight fever. One month later she developed slight pretibial edema, mild jaundice and considerable tenderness over the right upper abdominal quadrant. Death occurred Jan. 21, 1934.

Laboratory Findings: Urine: specific gravity 1.026, a trace of albumin, occasional hyaline casts. White blood count 20,900. Bromsulphalein retention 40 per cent at end of 30 minutes (2 mg. dosage). Van den Bergh reaction positive direct, serum bilirubin 1.32 mg. per 100 cc.

Autopsy Report

The autopsy was performed 1½ hours postmortem. The anatomical diagnoses were arteriosclerotic occlusion of the descending branch of the left coronary artery, and infarction of the interventricular septum and anterior and left lateral wall of the left ventricle

with extensive endocardial thrombosis. Emboli occluded the lumen of the celiac axis by two-thirds, and the right main branch of the hepatic artery completely, a short distance from its origin. Infarcts were present in the kidneys, lungs, spleen, stomach and liver.

The liver weighed 1310 gm. and was soft and friable with accentuated lobular markings. A pale, firm, slightly elevated, sharply demarcated area 7 by 5 by 2 cm., shaped like a truncated cone with its base towards the capsule, occupied a superficial position in the posterior inferior portion of the right lobe. On section the lesion was surrounded on all sides and beneath the capsule by a bright red, irregular serrated line (Fig. 2). The liver markings were absent throughout and the central portion of the necrotic area was darker and softer than the remainder, which was quite pale. A completely thrombosed branch of the hepatic artery extended directly into the lesion. The bile ducts and larger branches of the portal and hepatic veins were patent. The gall-bladder contained 30 cc. of dark, ropy bile.

Microscopic Examination

In the hepatic tissue away from the infarct there is a moderate degree of passive congestion, numerous areas of central focal necrosis and a slight degree of fatty change in some of the hepatic cells. The medial coat of the wall of the thrombosed branch of the hepatic artery is narrow and atrophied and the intima is markedly thickened by an eccentric, fibrotic and hyalinized plaque beneath which the internal elastic membrane is frayed. The lumen contains an adherent thrombus consisting of masses of fibrin, fused platelets, well preserved erythrocytes and leukocytes.

CASE 3. Phila. Gen. Hosp. 26368. F. N., a negro, aged 48 years, a chronic alcoholic, suddenly developed chills, cough, dyspnea, low blood pressure and pulmonary edema followed by a right-sided hemiplegia with fatal termination on Oct. 29, 1933. The quantitative estimation of sugar in the blood was 52 mg. per cent and of urea 50 mg. per cent.

Autopsy Report

The autopsy was performed 20 hours postmortem. The anatomical diagnoses were acute vegetative and ulcerative aortic endocarditis, diffuse suppurative myocarditis, embolic hemorrhagic abscesses

of the brain, embolism of branches of the hepatic artery, and infarcts of the liver, kidneys and spleen.

The liver weighed 1900 gm., was degenerated and studded throughout with numerous pale, wedge-shaped areas. The lobular markings were indistinct. The largest of these areas occupied a position along the lateral margin of the left lobe and was traversed by a completely occluded branch of the hepatic artery, which contained strands of fibrin, many leukocytes, erythrocytes and colonies of micrococci. The accompanying branch of the portal vein was patent.

CASE 4. Jeff. Hosp. D-1618. T. R., a white male, aged 64 years, was admitted to the hospital on June 16, 1930 with headache, fever, low blood pressure and marked infection of the jaws following extraction of teeth. There were small, red, tender nodules about several of the joints which were swollen and painful. The white cells of the blood ranged between 800 and 3900 with an average of 35 per cent polymorphonuclear leukocytes. There were 1,500,000 red blood cells and the hemoglobin was 30 per cent. The urine contained a trace of albumin and a few casts. No microorganisms grew in the blood culture. Death occurred on July 6, 1930.

Autopsy Report

The autopsy was performed 6 hours postmortem. The anatomical diagnoses were widespread petechiae of the skin, moderate diffuse subcutaneous edema, acute degeneration of the myocardium, congestion and edema of the lungs, chronic nephritis and senile arteriosclerosis of the abdominal portion of the aorta and its larger branches, and multiple infarcts of the spleen and liver.

The liver weighed 1200 gm., and showed passive congestion and central lobular coagulation necrosis, the smaller branches of the hepatic artery being markedly thickened. Scattered, small, sharply defined, grayish yellow areas not exceeding 3 cm. in width and softer in consistence than the surrounding tissue, were observed on the cut surface, especially beneath the capsule. The blood vessels were not explored but thrombosed branches of the hepatic artery, portal vein and hepatic vein were noted in direct relation to one of the areas of necrosis obtained for microscopic study; the thrombus in the branch of the hepatic artery was partially organized. The bile ducts were patulous and the gall-bladder contained a small amount of thin, dark green bile.

CASE 5. Phila. Gen. Hosp. 27587. A. B., a negro, aged 29 years, with enlargement of the heart and an apical systolic murmur died of pyemia on May 16, 1934, following incision and drainage of a perineal abscess, under local anesthesia.

Autopsy Report

The autopsy was performed 18 hours postmortem. The anatomical diagnoses were syphilitic aortitis with aneurysm formation, acute bacterial endocarditis of the aortic leaflets, focal embolic myocarditis, abscesses of the kidney and perineum, and septic thrombosis of small branches of the hepatic artery with multiple, small, pale infarcts of the liver.

The liver weighed 1700 gm. and showed portal venous congestion and periportal lymphocytic infiltration. There were several small, gray, firm, circumscribed areas 3-5 mm. in the superficial parenchyma. The blood vessels were not explored, but in the deepest portion of one of the infarcted areas obtained for microscopic section there were several necrotic vessels, one of which appeared to be a branch of the hepatic artery. These vessels were occluded and surrounded by masses of closely packed polymorphonuclear leukocytes and colonies of micrococci. Abscesses were present in the vicinity of the infarct.

COMBINED MICROSCOPIC EXAMINATION OF THE PALE INFARCTS IN CASES 2, 3, 4 AND 5

Within the areas of infarction there is complete coagulation necrosis with preservation of the architectural pattern, which is best maintained in the immediate vicinity of the portal radicles, whereas in the inner portion of the lobules autolytic changes are usually present to a slight degree. The reticulum is split, frayed and torn, and presents a thickened, lumpy, granular appearance, except along the outer rim of the lobules. The perivascular tissue spaces are edematous and the lumens of the sinusoids are usually empty or contain hematoidin burrs, a few heavy, rod-shaped bacteria and acidophilic granular debris with fragments of erythrocytes and leukocytes. Similar material is present in the Kupffer cells, which also share in the coagulative necrotic process. The lumens of the branches of the hepatic artery and portal and hepatic veins are either empty or filled to a variable degree with mottled thrombi or unrecognizable debris. Practically all the constituent cells of the portal

radicles are necrotic but the fibroblasts, bile duct epithelium and smooth muscle cells in the walls of the blood vessels are decidedly more resistant than the hepatic cells to the effects of ischemic changes.

The liver cords at the periphery of the infarct are directly continuous with others which usually form a sharply delimiting zone, 0.5-1 mm. thick around the area of complete necrosis (Fig. 3). This bordering zone presents as a rule a middle layer of coagulation necrosis merging on either side into layers of marked leukocytic infiltration, cellular disintegration, and sinusoidal thrombosis which is replaced by hyperemia as the normal liver tissue is approached. In the inner layer the necrotic material of the hepatic cells is cleared away by the action of macrophages and polymorphonuclear leukocytes, which penetrate the liver cords by way of the perivascular tissue spaces and sinusoids which are often outlined as basophilic smudges of necrotic phagocytes. The features of this bordering zone, which are most characteristic in Cases 2 and 3, are sometimes duplicated about some of the large sublobular veins, both within the areas of infarction and in the adjacent tissue.

COMBINED RED AND PALE INFARCT

CASE 6. Phila. Gen. Hosp. 24707. T. R., a negro, aged 37 years, with low blood pressure, weakness and excessive thirst, died in coma on Oct. 14, 1932. The urine had a specific gravity of 1029 and contained albumin, sugar and acetone.

Autopsy Report

The autopsy was performed 5 hours postmortem. The anatomical diagnoses were acute myocardial degeneration, bronchopneumonia, splenic enlargement, portal cirrhosis with fatty metamorphosis and multiple pale and red infarcts of the liver.

The liver weighed 1730 gm. and presented the picture of coarse, nodular, portal cirrhosis. On section there were a few, small, fairly well circumscribed necrotic areas. The bile ducts were patent and the gall-bladder contained 10 cc. of dark green bile. The vessels were not explored.

Microscopic Examination

Microscopically the liver is composed of varying sized nodular areas of hepatic cells showing congestion and an advanced degree of fatty change. The central veins are out of their usual positions and

the nodules are irregular in size and arrangement and are surrounded by broad bands of hyperemic, vascular connective tissue containing chronic inflammatory cells and proliferated bile ducts. In this connective tissue several large vessels which cannot be designated as arteries or veins contain thrombi composed of slightly basophilic granular material largely devoid of cells and fibrin. The changes in the areas of necrosis show evolution from red to pale infarction, being essentially similar to those already described. There is a marked tendency for the connective tissue septa to limit the spread of the necrotic process. In several areas of early involvement the reactive changes in the bordering zone are not intense and although the hepatic cells are necrotic, hemolysis has not advanced to any degree. The latter change is followed by the paling process, which begins in two or three small areas near the center of the nodule and spreads out peripherally. The abundant fat vacuoles appear to be equally numerous within and without the areas of infarction.

ORGANIZING INFARCT

CASE 7. Phila. Gen. Hosp. 24720. F. B., an insane female, aged 59 years, was subjected to cholecystostomy and incision and drainage of the pancreas under spinal anesthesia. Fat necrosis of the omentum and peripancreatic tissue was found, and death occurred one month later, Oct. 18, 1932. The urine contained sugar, albumin, and granular casts. The white cells in the blood were 27,200 per cmm.

Autopsy Report

The autopsy was performed 10 hours postmortem. The anatomical diagnoses were suppurative pancreatitis with fat necrosis of the omentum and peripancreatic tissue, sclerosis of the coronary arteries, acute myocardial degeneration, bronchopneumonia, generalized passive congestion and multiple infarcts of the liver.

The liver weighed 1170 gm., presented a nutmeg appearance, and contained a number of necrotic areas thought to be abscesses in the left margin of the left lobe. The blood vessels were not explored. The bile ducts were patulous and the mucosa of the gall-bladder was thickened and hemorrhagic.

Microscopic Examination

A section of one of these lesions contains an irregular area of hepatic infarction 1.3 cm. wide surrounded by connective tissue, ex-

cept beneath Glisson's capsule which is necrotic, markedly irregular, sunk below the general surface level of the liver and covered by an organizing peritoneal exudate. The changes in the necrotic area resembled those described under pale infarcts with certain additional features. The peripheral portion is powdered with chromatin particles of inflammatory cell nuclei in the sinusoids and contains hematin burrs and many acicular spaces, probably representing fatty acid crystals arranged in sheaves. The outer limits of this peripheral portion consist of a zone distinguished chiefly by the acidophilic staining reaction of its cytoplasmic masses, and the presence of long slender projections of newly formed connective tissue and capillaries penetrating at right angles from the capsule surrounding the lesion on all sides. This capsule of the infarct (Fig. 4) consists of actively organizing connective tissue infiltrated by lymphocytes, pigmented monocytes and proliferating tubular structures originating mainly from the portal radicles on its outer aspect. The tubules are round, oval, elongated and irregularly branched structures, usually with a lumen and lined by small, darkly stained cuboidal or flattened epithelial cells with hyperchromatic nuclei, but with no mitotic figures. The branches of the hepatic artery and portal vein in the portal radicles immediately outside the capsule are completely effaced by connective tissue in many instances. One large vessel which cannot be identified as either artery or vein contains an organized, canalized thrombus. The parenchyma lying adjacent to the capsule of the infarct shows dilated sinusoids, pigmentation and regeneration. Some of the arterial branches throughout the remainder of the liver show marked intimal thickening with consequent diminution in the diameter of the lumens.

POSSIBLE HEALED INFARCT

CASE 8. Phila. Gen. Hosp. 26506. A. F., a white male, aged 63 years, died with a left-sided hemiplegia on Nov. 20, 1933. Examination of the blood and spinal fluid disclosed no evidence of syphilis.

Autopsy Report

The autopsy was performed 10 hours postmortem. The anatomical diagnoses were coronary sclerosis with old and recent myocardial infarction and endocardial thrombosis, generalized passive congestion, encysted thoracic empyema, a small peritoneal abscess,

marked atheroma with ulceration of the aorta, senile atrophy of the kidneys, possible areas of mesenteric thrombosis, recent splenic infarction and an area of possible healed hepatic infarction.

The liver weighed 1600 gm. and presented the picture of advanced chronic passive congestion with marked fatty change. On the anterior aspect of the inferior border of the right lobe there was a firm, white, depressed nodule, measuring approximately 1 cm. in diameter. The bile ducts were patent and the gall-bladder was distended with thin, brownish red bile.

Microscopic Examination

Histological sections of the nodule disclose a roughly rectangular area continuous with Glisson's capsule, submerged slightly below the general surface level of the liver and set off sharply from the hepatic parenchyma on the remaining three sides by a zone of proliferated tubular structures. It is composed of whorls and interlacing bands of comparatively acellular collagen fibrils laid down along the lines of the previous pattern of the liver (Fig. 5). The lumens of most of the sinusoids are obliterated by fine collagen fibrils, in contrast to the more densely arranged hyalinized connective tissue which has replaced the hepatic cords. Some of the sinusoids are partially patent, lined by endothelial cells, and contain normal red blood cells, monocytes, lymphocytes and polymorphonuclear leukocytes, rarely exceeding ten in number in any single sinusoid. Other structures are difficult to designate with any degree of certainty. The bile ducts are completely effaced, occasional sublobular veins are thrombosed, and a few arterial branches are thickened, usually to the point of complete occlusion. There are many small patent vessels in some of the portal radicles. This area is surrounded on three sides by a wide zone of proliferating tubular structures resembling small bile ducts and supported by loosely arranged, relatively avascular connective tissue infiltrated by lymphocytes and studded with remnants of several portal areas containing occluded hepatic arterial branches. A large portal area just beyond one corner of this outer zone contains a thrombosed canalized branch of the hepatic artery accompanied by a patent branch of the portal vein (Fig. 6). Similar arterial changes are noted in other portal areas in relation to the lesion. The branches of the arteries and veins elsewhere in the parenchyma show no thickening.

TABLE I

Reports of Hepatic Infarction

Author	Distribution in liver	Hepatic vascular lesions	Background
Chiari, H. (Case 21)	Necrosis of entire liver	Embolism of hepatic artery	Acute and chronic mitral endocarditis
Chiari, H. (Case 22)	Multiple small infarcts	Embolism of smaller branches of hepatic artery	Acute and chronic mitral endocarditis
Baldwin, F. A.	Multiple small pale infarcts with beginning organization	Thrombosis of smaller branches of hepatic artery	Aortic stenosis and regurgitation, thrombosis of right auricle, chronic passive congestion
Ruczyński, B. (Case 1)	Multiple small infarcts in right lobe	Embolism of branches of hepatic artery, more recent thrombosis of portal and hepatic veins	Vegetations in right ventricle, arteriosclerosis with ulceration and thrombus formation of the aorta
Beresnegowski, N.	Multiple small infarcts in right lobe	Surgical ligation of right branch of hepatic artery, more recent thrombosis of portal vein	Operation for carcinoma of gall-bladder
Narath, A.	Necrosis of entire left lobe, Spiegelian lobe and part of right lobe	Surgical ligation of hepatic artery	Gastric resection for carcinoma of stomach
Wendel, W. Kretz, R.	Almost total necrosis of liver	Surgical ligation of hepatic artery	Operation for carcinoma of stomach
Askauazy, M.	Multiple large infarcts in both lobes	Embolism of smaller branches of hepatic artery	Acute endocarditis
Mittasch, G.	Multiple infarcts	Embolism of smaller branches of hepatic artery	Arteriosclerosis with ulceration and thrombus formation of the aorta, operated upon for mesenteric thrombosis
Orlandi, N. (Case 1)	Multiple small infarcts of right lobe	Obliterating endarteritis of hepatic artery and embolism of smaller branches	Mitral stenosis with thrombi in auricles, chronic passive congestion, arteriosclerosis
Orlandi, N. (Case 2)	Multiple small infarcts in both lobes	Embolism of smaller branches of hepatic artery, more recent thrombosis of portal vein branches	Chronic mitral endocarditis, mural thrombi in left ventricle
Orlandi, N. (Case 3)	Multiple small infarcts, small embolic abscesses	Embolism of smaller branches of hepatic artery, more recent thrombosis of portal vein branches	Vegetative mitral and tricuspid endocarditis
			Osteomyelitis, pyemia with "foci" on aortic valve leaflets, chronic passive congestion

Orlandi, N. (Case 4)	Multiple infarcts in right lobe	"Primary" thrombosis of portal vein branches, "secondary" thrombosis of smaller branches of hepatic artery	Calculus cholecystitis with perforation of gall-bladder and peritonitis
Orlandi, N. (Case 5)	Multiple infarcts	"Primary" thrombosis of portal vein branches, "secondary" thrombosis of smaller branches of hepatic artery	Pylephlebitis with pylethrombosis, acute gastritis, "initial cirrhosis"
Cioni, C. (Case 1)	Large infarct in right lobe	Embolism of right branch of hepatic artery	Acute mitral and tricuspid endocarditis, mural thrombosis in left auricle, arteriosclerosis, "increase of periportal connective tissue," chronic passive congestion
Cioni, C. (Case 2)	Multiple large and small infarcts in left lobe	Septic thrombosis of left branch of hepatic artery, beginning thrombosis of portal vein	Gastric ulcer with resection and gastro-enterostomy, acute peritonitis
Graham, R. D., and Cannell, D.	Multiple large and small infarcts in left lobe	Surgical ligation of hepatic artery	Partial resection of stomach and gastrojejunostomy for carcinoma
Shann, H., and Fradkin, W. Z.	Single large infarct in right lobe with sequestration	Surgical ligation of hepatic artery	Cholecystectomy for calculous cholecystitis
Kerr, R. W.	Multiple infarcts in right lobe	Ligation of portal vein together with right branch of hepatic artery	Cholecystectomy
Lund, H., Stewart, H. L., and Lieber, M. M. (Case 1)	Large red infarct in left lobe	Embolism of large branch of hepatic artery	Acute vegetative endocarditis, septicemia
Lund, H., Stewart, H. L., and Lieber, M. M. (Case 2)	Large pale infarct in right lobe	Embolism of large branch of hepatic artery	Arteriosclerotic occlusion of left coronary artery with myocardial infarction and endocardial thrombosis
Lund, H., Stewart, H. L., and Lieber, M. M. (Case 3)	Multiple small pale infarcts	Embolism of smaller branches of hepatic artery	Acute vegetative and ulcerative endocarditis
Lund, H., Stewart, H. L., and Lieber, M. M. (Case 4)	Multiple small pale infarcts	Thrombosis of smaller branches of hepatic artery and portal and hepatic veins	Marked oral sepsis following extraction of teeth, aplastic anemia, aortic arteriosclerosis
Lund, H., Stewart, H. L., and Lieber, M. M. (Case 5)	Multiple small pale infarcts	Embolism of smaller branches of hepatic artery, thrombosis of branches of portal and hepatic veins	Acute bacterial endocarditis, pyemia
Lund, H., Stewart, H. L., and Lieber, M. M. (Case 6)	Multiple small pale and red infarcts	Thrombi in small vessels not identified as arteries or veins	Portal cirrhosis of liver
Lund, H., Stewart, H. L., and Lieber, M. M. (Case 7)	Multiple small organizing infarcts in left lobe	Organized, canalized thrombus in large vessel not identified as artery or vein	Suppurative pancreatitis, cholecystostomy with incision and drainage of the pancreas

DISCUSSION

It is generally agreed that the life of the hepatic cell is dependent upon an adequate supply of arterial blood and is affected only to a relatively slight degree by diminution in the portal blood supply. Segall has demonstrated that necrosis of the liver results from more or less sudden total occlusion of the main stem of the hepatic artery in the absence of accessory arterial twigs or of unusually large collateral branches. Because of abundant collaterals between the right and left main branches, obstruction of either of these arteries leaves the liver in its normal condition. Those intrahepatic branches that have subcapsular ramifications necessarily have collaterals through the surface anastomoses with branches of the phrenic arteries. The frequency of occurrence and variability in distribution of these anomalous and collateral vessels preclude the possibility of generalizing in regard to the probable effects of interfering with the blood flow at any particular point in the hepatic arterial tree. However, the arterial branches which end within the liver substance and do not take any part in the subcapsular ramifications have only an extremely limited course of collateral circulation from the anastomosing small vessels in the portal sheath and around the portal vein, bile ducts and nerves; hence, obliteration of these vessels, properly designated as end-arteries, will be followed by infarction.

Occlusion of the hepatic artery or its branches was present in 25 of the 27 cases of this series; in the other 2 the vessels were not explored grossly and the thrombosed branches could not be identified as arteries or veins in microscopic sections. The interruption of arterial blood flow was considered primarily responsible for the infarction in 23 cases, in 9 of which there was secondary occlusion of the accompanying branches of the portal vein. In Orlandi's fourth and fifth cases portal vein occlusion was thought to have preceded thrombosis of the arteries. The importance of the relation of the blood vessels of the liver to hepatic infarction is clearly demonstrated in Kerr's case, in which the main branch of the portal vein and right branch of the hepatic artery were ligated, the areas of infarction being confined to the right lobe of the liver. The mechanism of hepatic artery occlusion was considered to be embolic in 14 cases, thrombotic in 5, and surgical ligation in 6. Emboli probably arose from thrombotic masses in the heart and aorta. The possible etio-

logical factors responsible for thrombosis of the hepatic artery were arteriosclerosis, cholecystitis, peritonitis, gastric ulcer, propagation of thrombi from the celiac axis, pancreatic necrosis and surgical procedures about the biliary and gastro-intestinal tracts.

Three cases of hepatic infarction occurred in cirrhotic livers. Cioni's case was due to embolism of the hepatic artery from the lesions of acute endocarditis. Orlandi's case was associated with an incipient cirrhosis, acute gastritis, pylethrombosis and secondary occlusion of the hepatic artery. In our own case of typical portal cirrhosis in the active stage, the background for the development of infarction is obscure and the large thrombosed vessels noted in histological sections could not be identified as either venous or arterial branches. Kaufmann and Rolleston and McNee believe that obliteration or thrombosis of the portal vein in cirrhotic livers may lead to the production of necrotic lesions comparable to infarcts. This seems improbable in view of the fact that in advanced cases of portal cirrhosis the condition of the hepatic circulation is comparable to that which exists following the production of an Eck fistula, and the task of supplying sufficient blood to the parenchyma for normal metabolism is relegated to the hepatic artery (McIndoe). Complete occlusion of the portal vein under these circumstances would hardly lead to infarction of the liver.

The retardation of autolytic processes within infarcted tissue in the liver resembles that in infarcts of the kidney and spleen. Orth stated that following a primary imbibition of extravasated plasma, the infarcted areas undergo a dehydration process and are slowly lysed in the course of weeks or months by the action of invading leukocytes. Although autolytic changes in the nuclei of the affected cells occur relatively early, the inhibition of total autolysis is due to the fact that the alkaline plasma, seeping into the necrotic tissue, furnishes a poor medium for the action of autolytic enzymes, but favors the action of heterolytic enzymes contained in the leukocytes along the margin of the lesion (Wells). The explanation of this retardation seems to lie in the relation between the proteolytic enzyme, cathepsin and its activator sulphhydryl, which is a reduction product of glutathione in its disulphide form. Borger, Peters and Kurz found that the concentration of reduced glutathione became rapidly diminished in areas of infarction experimentally produced, indicating that instead of the disulphide being reduced to the sulphhydryl it is

changed to irreversible oxidation products, the oxidation being accelerated by the alkaline reaction. With the disappearance of sulphydryl, a strong enzymatic action leading to liquefaction can, therefore, hardly take place.

The relation between fatty changes in necrotic and non-necrotic tissue of infarcted livers has been commented upon by Bainbridge and Leathes, and Cameron and Mayes, who noted a definite increase in the cellular content of free fat in lesions produced by ligation of the hepatic artery in animals. In our cases, fat droplets and vacuoles indicative of fat appeared to be identical in the area of infarction and surrounding tissue in the early stage of the lesion (Cases 1 and 6); later on (Case 2) there was a concentration of free fat along the margin which in the late organizing infarct (Case 7) contained many acicular spaces of fatty acid crystals. The explanation for the deposition of lipoidal substances in the macrophages (Ribbert), and elsewhere about the periphery of infarcts (Fischler), is assumed to lie in the persistence of cell lipase which synthesizes fatty acid and glycerol diffusing into the necrotic area with the plasma, unchecked by normal oxidative destruction of these substances (Wells).

Evidences of regeneration were present in the hepatic parenchyma in the vicinity of all the infarcted areas of our cases. These were characterized by hypertrophy and hyperchromasia, and by nuclear fission and budding, resulting in the formation of binuclear and multinuclear hepatic cells. No mitotic figures were observed. Although regeneration was regularly more active about the portal radicles than in the remaining portions of the lobules, this can probably be accounted for by the better blood supply in these areas and is not necessarily indicative of the origin of regenerating hepatic cells from bile duct epithelium. Tubular structures resembling bile ducts, and showing at first no connection with hepatic cells, grew out from the portal radicles in the viable parenchyma adjacent to the capsule of the organizing infarct. In the case of possible healed infarct these structures seemed to be continuous in some instances with the cords of liver cells in the surrounding parenchyma but did not give rise to hepatic cells within the lesion itself.

The sequence of events leading to the ultimate disposure of infarcted areas in the livers of human beings is poorly understood because the complicating factors in these cases usually precipitate an early fatal termination. In animals subjected to ligation of the he-

hepatic artery the necrotic lesions sometimes undergo abscess formation and calcification. In human beings large infarcted areas may become sequestered (Shann and Fradkin), but do not appear to eventuate in abscess formation, despite the fact that they are produced by septic emboli in some instances, embolic abscesses being present in their immediate vicinity and elsewhere in the liver. The only example of possible healed infarct reported in the literature is that by Rattone. In our case we feel that the evidence favors healed infarction because of the relation of the canalized thrombosed branch of the hepatic artery to the lesion and the preservation of the architecture in the area of involvement in the presence of complete replacement by connective tissue. It is logical to assume that had the lesion resulted from abscess formation the structural pattern would have been destroyed, and if due to tuberculosis the lesion would have had more specific characteristics. The negative blood and spinal fluid Wassermann, negative colloidal gold curve, and absence of other evidences of syphilis, all argue against the possibility of gumma. The question of tumor formation does not seem to enter into the differential diagnosis.

Jaundice was present in 7 cases of this series of hepatic infarction. Factors other than the infarct itself were probably responsible for its production, since the secretory and excretory functions of the liver can be adequately maintained by a very small amount of normal hepatic parenchyma. Factors contributing to the production of hyperbilirubinemia in this condition appear to be myocardial insufficiency, infection, pulmonary infarction and cholecystitis in association with diffuse regressive lesions in the hepatic parenchyma. It is remarkable that Chiari made no mention of jaundice, although he reported total infarction of the liver. Following the production of necrotic hepatic lesions by experimental ligation of the hepatic artery in animals Betz, Asp, and Cameron and Oakley noted no disturbance in bile formation or excretion. In Graham and Cannell's collected series of 28 cases of accidental ligation of the hepatic artery in human beings jaundice was mentioned in five instances. No complicating factor was evident in Kehr's case but in the others there were calculous cholecystitis (Smith), terminal peritonitis (Ritter), hepatic traumatism (Behrend), and rupture, multiple abscesses and sequestration of the liver (Sprengle).

SUMMARY

Infarcts of the liver may be single or multiple and generally occupy a superficial position beneath the capsule unless the area of involvement is coextensive with a whole lobe or the entire organ, a large part of which may undergo sequestration in exceptional instances. In the early stages the lesion is usually firm, red, elevated and sharply demarcated by an irregular wavy line of vascular congestion which fades gradually into the surrounding parenchyma. In the presence of portal cirrhosis the area of infarction seems relatively less firm and is limited by the coarser bands of connective tissue. Otherwise the extent of the necrosis depends roughly, in the absence of collaterals, on the distribution of the branch of the hepatic artery occluded by ligatures, emboli in the systemic circulation and thrombi resulting from infection or trauma sustained in operative procedures upon the biliary system or gastro-intestinal tract. On section the deeper border of the infarct is indented along the lines of exit of the branches of the hepatic veins and is sharper palpably than visually, although the markings of the liver are usually totally effaced in the area of involvement. A layer of viable tissue is regularly maintained immediately beneath the capsule of the liver in the early stages and assumes the appearance of rete pegs where the collateral vessels of the capsule anastomose with those in the superficial parenchyma. In the pale infarct the center may be softer and darker, indicating apparently that the hemoglobin has greater difficulty in escaping from this region, despite the fact that hemolysis seems to occur here first. The red color of the entire necrotic area may persist even after hemolysis is practically complete, owing to the diffuse staining by hemoglobin which escapes chiefly by plasmatic diffusion, the remainder being broken down into granular and fine needle-shaped crystalline pigment. Parenchymal necrosis is evident before hemolysis has occurred to any extent and, although the sinusoids are packed with red blood cells, there is little or no tendency for hemorrhage to occur into the tissue surrounding vascular structures.

The delimiting zone of an infarct is characterized at first by sinusoidal congestion and by an infiltration of phagocytic cells which rapidly increase in number. Ultimately this reactive border and a narrow strip of adjacent parenchyma, including the surface of the liver along the superficial aspect of the lesion, succumb to complete

necrosis and sinusoidal thrombosis. A thick capsule of organizing fibrous tissue then forms around the outer limits of the necrotic tissue, which is invaded only slowly by the proliferating capillaries and fibroblasts. Tubular structures resembling pseudobile canaliculi proliferate from the portal radicles in the parenchyma bordering the capsule of the infarct. Subsequently, as judged by the findings in 2 cases of possible healed infarction, the connective tissue invading the area of necrosis proceeds along the lines of the previous pattern of the liver and becomes decidedly denser in the areas formerly occupied by the liver cords and sinusoids, the normal architecture being closely duplicated with ultimate and complete organization of the necrotic area. The tubular structures proliferating on the parenchymal side of the capsule are continuous sometimes with the hepatic cells of the liver cords, but do not give rise to hepatic cells within the thick wall which they finally form around the central fibrous area.

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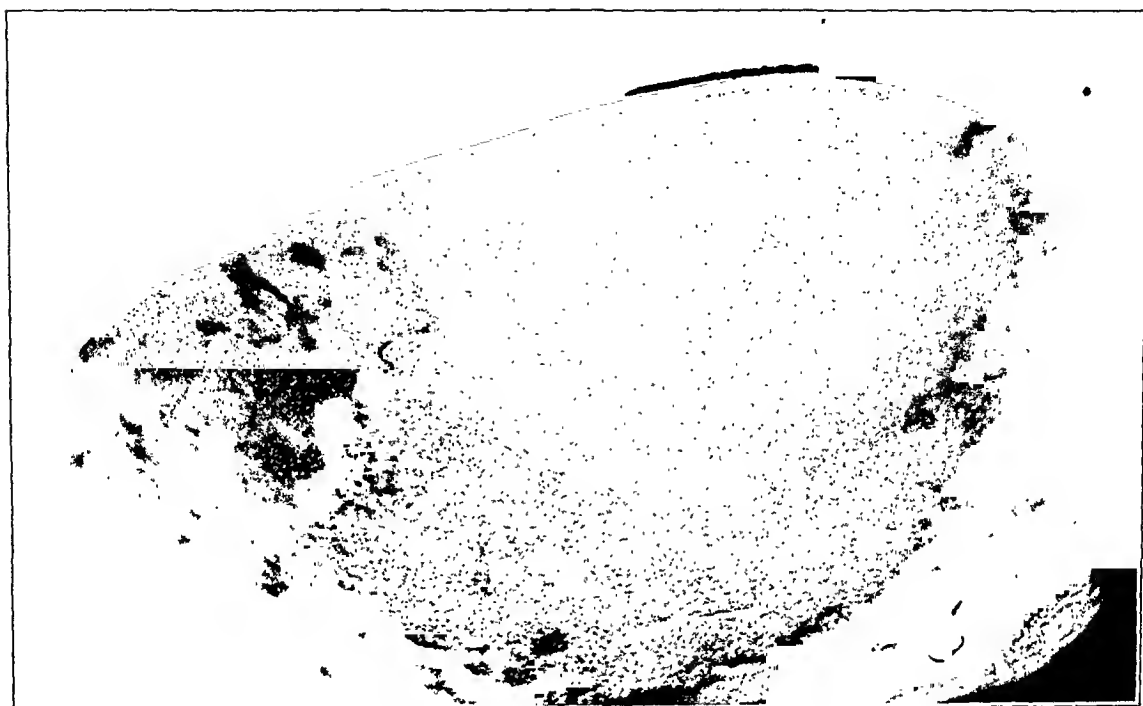
DESCRIPTION OF PLATES *

PLATE 21

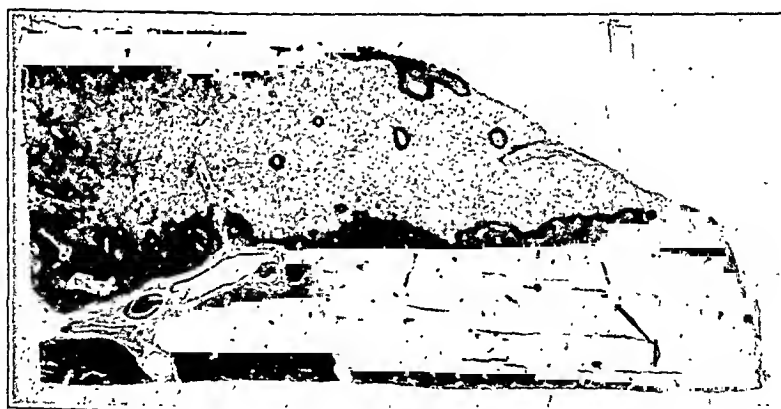
FIG. 1. Case 1. Red infarct. The lesion is mottled, slightly elevated and sharply demarcated from the surrounding hepatic tissue. Photograph of gross specimen.

FIG. 2. Case 2. Pale infarct. The capsule of the liver is intact in the upper portion of the illustration, but is torn away along the right upper border. A large portal area containing a patent branch of the portal vein and two thrombosed branches of the hepatic artery lies in relation to the lower left margin of the infarcted area. Hematoxylin-eosin stain. $\times 2$.

* An excellent reproduction of Mr. H. J. M. Nieuwenhuis' drawing of an area of anemia infarction in the right lobe of the liver following ligation of the right branch of the hepatic artery is depicted in the Atlas of Selected Cases of Pathological Anatomy by W. M. deVries, J. H. DeBussy, Ltd., Amsterdam, 1933, Plate 37.



I

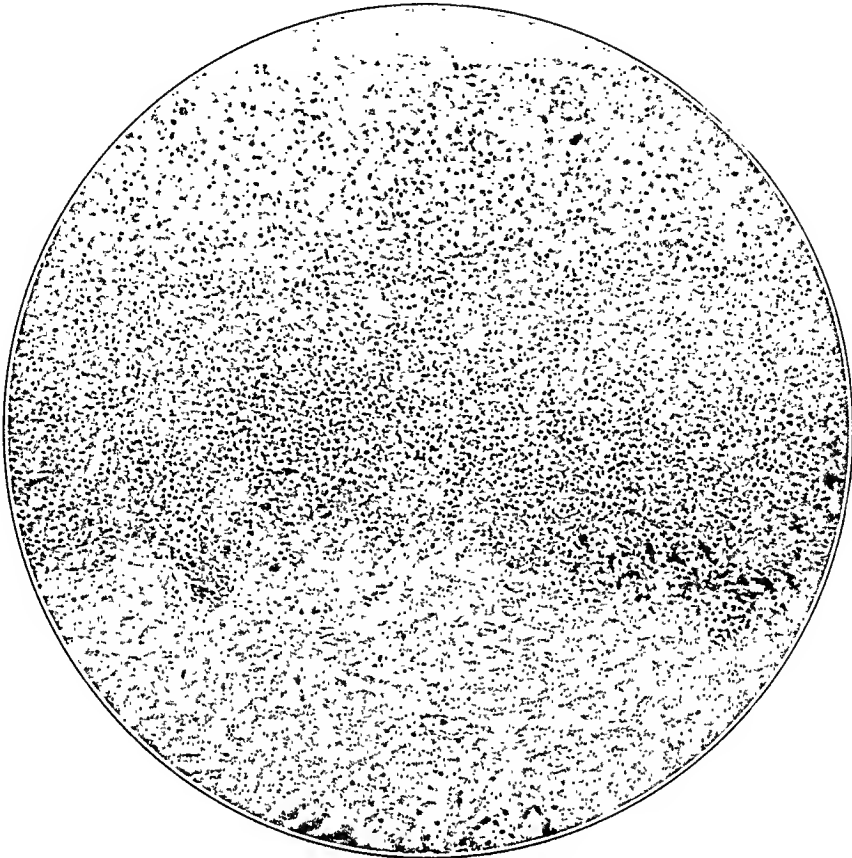


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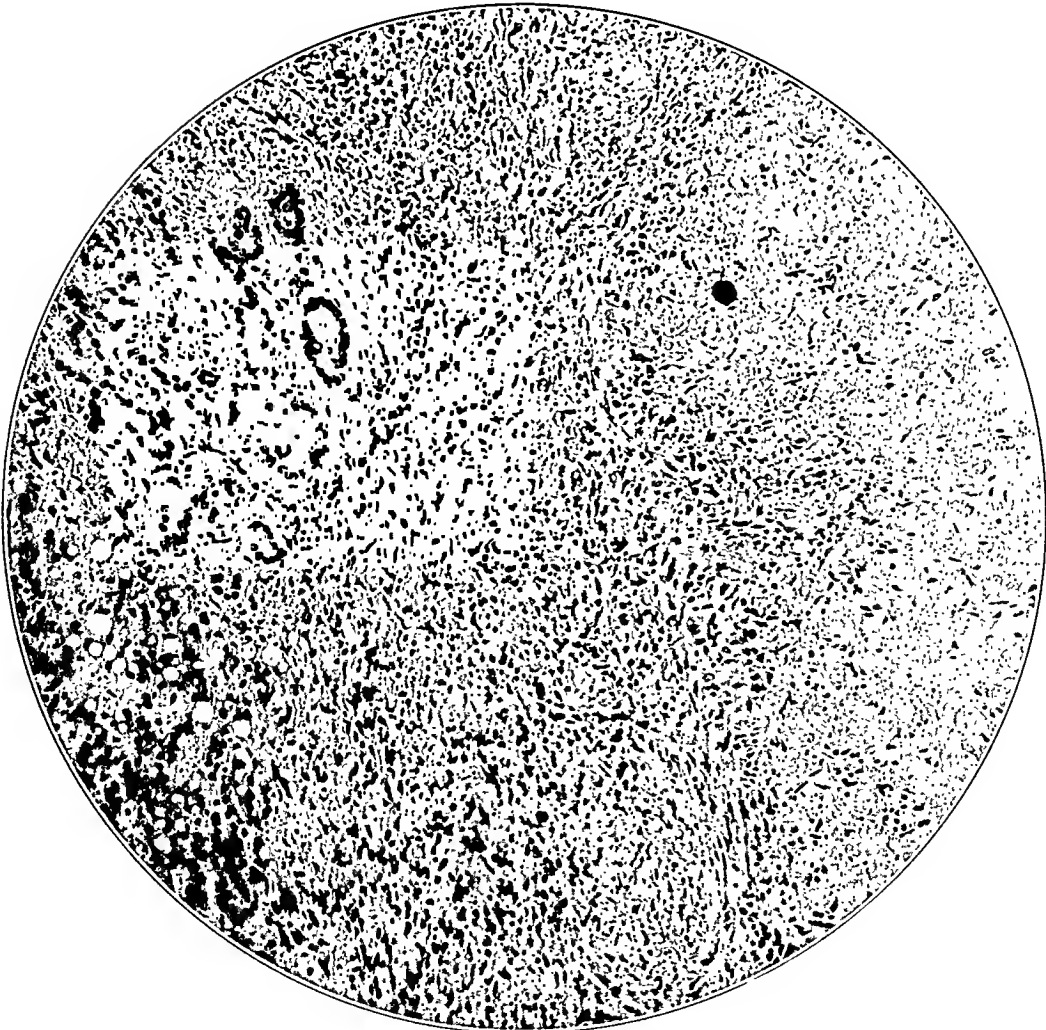
PLATE 22

FIG. 3. Case 2. Bordering zone of infarct. The capsule of the liver with a few underlying viable hepatic cells may be seen in the upper portion of the illustration. Note the sharp line of demarcation between the zone of inflammatory cell infiltration and the area of coagulation necrosis below. $\times 100$.

FIG. 4. Case 7. Encapsulated portion of organizing infarct showing necrotic tissue above and viable parenchyma at bottom of illustration. Note the proliferating tubular structures along the outer aspect of the capsule and the invasion of the necrotic margin by granulation tissue. $\times 100$.



3

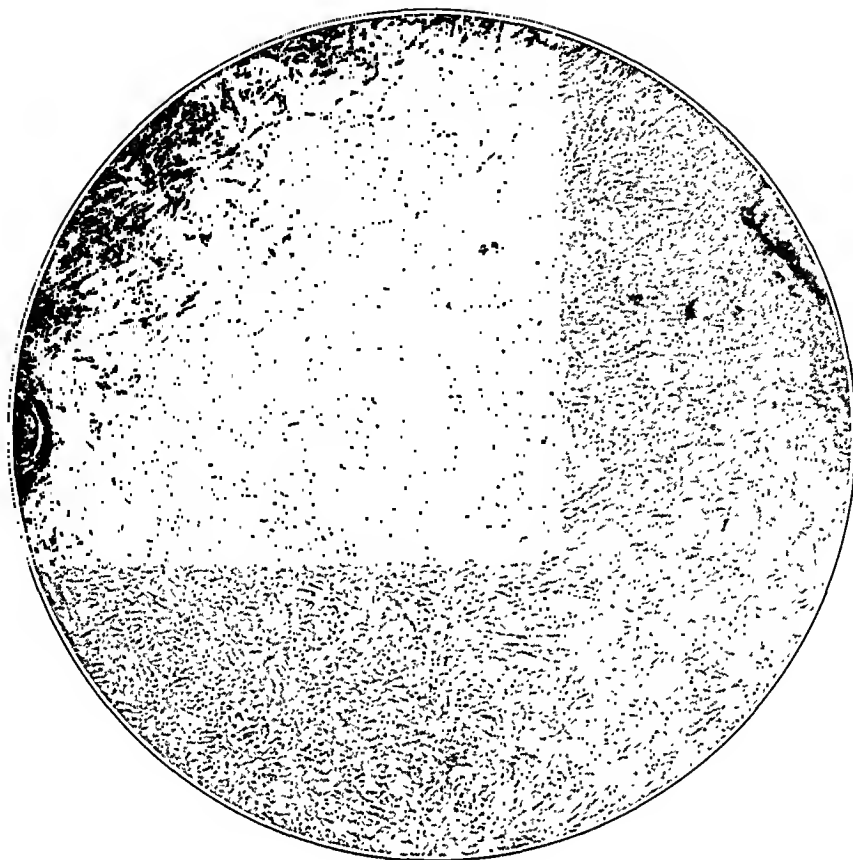


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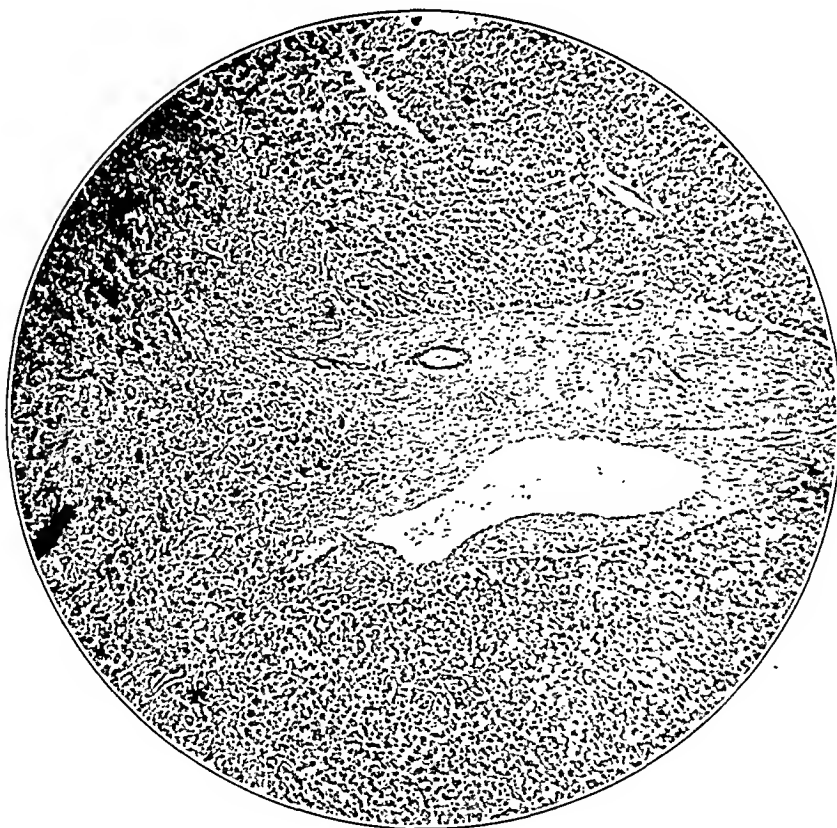
PLATE 23

FIG. 5. Case 8. Central area of possible healed infarct. The interlacing strands of connective tissue suggest liver cords and sinusoids. Portions of two large portal areas are present along the lateral margins of the illustration. Verhoeff's elastic tissue stain. $\times 50$.

FIG. 6. The margin of the lesion shown in Fig. 5 may be seen in the left upper quadrant of the illustration. A large portal radicle containing a patent branch of the portal vein and a canalized, organized thrombosed branch of the hepatic artery leads directly into the lesion. $\times 50$.



5



6

ANNULAR PANCREAS *

REPORT OF A CASE, WITH A SIMPLE METHOD FOR VISUALIZING THE DUCT SYSTEM

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The term "annular pancreas" or "ring pancreas" is applied to a comparatively rare developmental anomaly in which the second portion of the duodenum is encircled by a ring of pancreatic tissue. In 1933 one of us¹ reported a case of annular pancreas and summarized 39 other cases from the available literature. The embryology of the pancreas and the possible explanations for the ring portion were reviewed and illustrated, the cases were tabulated and an extensive bibliography listed. In most of the carefully described cases there was constriction of the duodenum with varying degrees of dilatation of the proximal portion of the small bowel, pylorus and stomach. Signs and symptoms of high intestinal obstruction were frequently reported, hence this anomaly must be considered in cases presenting such symptoms. The fact that Smetana² reported an annular pancreas in a man 74 years of age shows that it is possible to live the normal span of life with this anomaly. This patient, however, died following a gastro-enterostomy performed to relieve a progressive duodenal obstruction caused by the pancreatic ring.

We have a twofold purpose in writing this paper; first, to record another case of annular pancreas, and second, to call attention to a rapid and simple method of accurately tracing the pancreatic ducts.

In addition to the literature summarized in 1933, we have noted 3 more reports of annular pancreas.^{3, 4, 5} The following case brings the total number of recorded cases to 44.

REPORT OF CASE

Clinical History: A white male, 70 years of age, noticed an enlarging painless mass in the side of his neck for 8 months. He had previously been well except for

* Supported in part by the Rockefeller Fluid Research Fund of the School of Medicine of Stanford University.

Received for publication August 7, 1934.

an illness diagnosed as coronary occlusion 3 years before, and mild diabetes recognized for 3 years.

Physical examination showed an obese, aged man with a large, tender, infiltrating tumor in the left side of the neck. The heart was enlarged, but there were no other abnormal physical findings.

Laboratory tests were normal. The urine was sugar-free, with a number of leukocytes and an occasional hyaline cast in the sediment. The blood sugar ranged from 96 to 163 mg. per cent.

The neck tumor increased rapidly in size and became very painful. Death followed progressively increasing weakness.

AUTOPSY REPORT

Autopsy, No. xxxvii-105, performed 4 hours after death, showed the body of an obese male about 70 years of age with a firm left cervical tumor 15 cm. in diameter which infiltrated the skin and muscle. Both axillae contained walnut-sized lymph nodes. Many mediastinal lymph nodes were infiltrated with white tumor and a large mass of similar tissue surrounded the upper trachea. The heart was slightly enlarged. The descending branch of the left coronary artery was occluded and there was an old scar at the apex of the left ventricle. The lungs showed extensive bronchopneumonia and pleurisy. The spleen contained small tumor nodules and there was a small accessory spleen in the mesentery. The kidneys were fused to form a typical horseshoe kidney measuring 29 by 5.5 by 3.5 cm. The right renal pelvis contained several small stones. There were a few, small, gray tumor nodules beneath the liver capsule. The stomach was normal. A flat band of pancreatic tissue 2.5 cm. broad and 0.6 to 0.8 cm. thick completely encircled the second portion of the duodenum (Fig. 1). This tongue of tissue projected to the right from the head of the pancreas posteriorly, encircled the duodenum, and again fused with the anterior portion of the head in its mid-portion. Anteriorly the ring was somewhat flattened over the terminal branches of the superior pancreaticoduodenal artery. The duodenum was not constricted by the ring of pancreatic tissue and there was no appreciable dilatation above it. The duodenal papilla lay in the usual position. The pancreatic duct of Wirsung measured 0.6 cm. in circumference and entered the ampulla of Vater with the bile duct. The accessory pancreatic duct of Santorini measured 0.7 cm. in circumference and opened in the left anterior wall of the duodenum 1.5 cm. above the duodenal papilla. The two ducts were united in the normal manner. A moderately large duct arose in the

left anterior tip of the ring portion, circled the duodenum to the right and posteriorly with increasing caliber, and joined the pancreatic duct 2.25 cm. from the duodenal orifice (Fig. 2, DA).

MICROSCOPIC EXAMINATION

Histological examination of the organs shows the tumor to be a lymphosarcoma. Sections of the kidney and accessory spleen are normal, except for arteriosclerotic changes. Sections from various portions of the pancreas are normal and islands of Langerhans are plentiful. One island in the tail is about five times normal size.

Anatomical Diagnoses: Lymphosarcoma involving cervical, axillary and mediastinal lymph nodes, spleen, liver and skin; bronchopneumonia; acute fibrinous pleurisy; generalized arteriosclerosis; old coronary thrombosis with infarction of the heart; thrombosis of the left ventricle and the periprostic veins; renal calculi; biliary calculi; mild chronic cholecystitis; hypertrophy of the prostate; and the following congenital malformations: horseshoe kidney, accessory spleen and annular pancreas.

GENERAL DISCUSSION

Embryologically, the human pancreas arises as two distinct entodermal outgrowths, the dorsal and ventral anlagen, on opposite sides of the intestinal tube. As each elongates, rotation causes the ventral anlage to approach and unite with the dorsal. The dorsal anlage is large and grows across the body until it reaches the spleen. It forms the tail, the body and the ventral portion of the head of the adult gland. Its duct opens into the duodenum above the duodenal papilla but usually anastomoses with the ventral duct, which ends close beside the common bile duct in the ampulla of Vater. The ventral anlage forms a part of the head and the uncinat process of the pancreas. The ventral duct by an anastomosis with the duct of the dorsal pancreas becomes the outlet of the pancreatic duct of Wirsung. It will be noted that a large part of the dorsal pancreatic duct extending through the tail and body becomes incorporated in this main duct of Wirsung and the original outlet of the dorsal duct often atrophies but may remain functional as the accessory pancreatic duct of Santorini. It is through accurate knowledge of the arrangement of the ducts in the annular pancreas that its origin has been

traced to an anomaly of the ventral pancreatic anlage. The annular pancreas differs from the normal gland only in the ring portion, which arises from the dorsal portion of the head of the pancreas, and the ducts are comparable to those of the normal pancreas. Lecco⁶ believes that the tip of the ventral anlage becomes adherent to the duodenal wall so that in the normal rotation and migration this portion of the pancreas is stretched to form the ring.

Several methods have been suggested for tracing the ducts of the pancreas, but none has proved entirely satisfactory in our hands. The ducts may be injected with a dye such as methylene blue or with mercury to facilitate sharp dissection, but either is a messy procedure and often large ducts are unexpectedly cut and true relations lost. We suggest a simple and accurate technique for demonstrating the duct system without damage to the specimen.

INJECTION TECHNIQUE

Remove the intact pancreas and duodenum from the body. Cut across the tail of the pancreas and insert a small glass cannula into the tiny central duct, which will be easily found (Fig. 2, c). The cannula should be anchored by a ligature around the tail of the pancreas. Connect the cannula by means of rubber tubing either to a 20 cc. glass syringe, as used by Hill⁷ for the injection of blood vessels, or to an injection system as described by Poth.⁸ The injection material is an aqueous bismuth-acacia cream consisting of 10 per cent powdered acacia dissolved in boiling water, to which is added 20 per cent finely ground bismuth oxychloride. The mixture, when poured through a closely meshed cloth to remove the larger aggregates, is ready for use. The cream is placed in a syringe or pressure pump connected with the cannula in the duct of the tail of the pancreas and slowly injected. When the white mixture appears at the duodenal papilla, clamp and tie the tip, likewise the end of the accessory duct, if patent, and any other points of leakage. The greater the pressure of the injection, the more complete will be the filling of the smaller branches of the ducts. Remove the cannula and tighten the ligature. The radio-paque bismuth suspension clearly outlines the duct system when viewed through the fluoroscope. Place the organ in such a position that the course of the ducts is readily demonstrated and make a roentgenogram for a permanent record. Stereoscopic films are valu-

able if the duct system is complicated. The results of an injection by the Poth⁸ technique are shown in Figure 2. The specimen may be fixed, sectioned and stained as usual. The bismuth will be black in the stained sections.

DISCUSSION OF THE CASE

Through stereoscopic roentgenographic studies of the course of the ducts in our case of annular pancreas it is evident that the ring portion had its origin in the ventral pancreatic anlage. The duct of the ring opened into the main pancreatic duct close to the ampulla of Vater and was only indirectly connected with the portion of the duct which developed in the dorsal anlage.

This patient lived to the age of 70 years with no complaints referable to his annular pancreas or other congenital anomalies. Twenty-five per cent of the reported cases of annular pancreas are associated with other congenital anomalies.

SUMMARY AND CONCLUSIONS

1. Another case of annular pancreas is recorded, bringing the total number of reported cases to 44.
2. Annular pancreas is undoubtedly a developmental anomaly of the ventral pancreatic anlage.
3. Our case was that of a man 70 years of age with several congenital anomalies but with no complaints referable to them.
4. A rapid and simple method of accurately tracing the duct system of the pancreas is described.

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DESCRIPTION OF PLATES

PLATE 24

FIG. 1. Specimen from the case reported, showing the anomalous ring of pancreatic tissue encircling the duodenum.

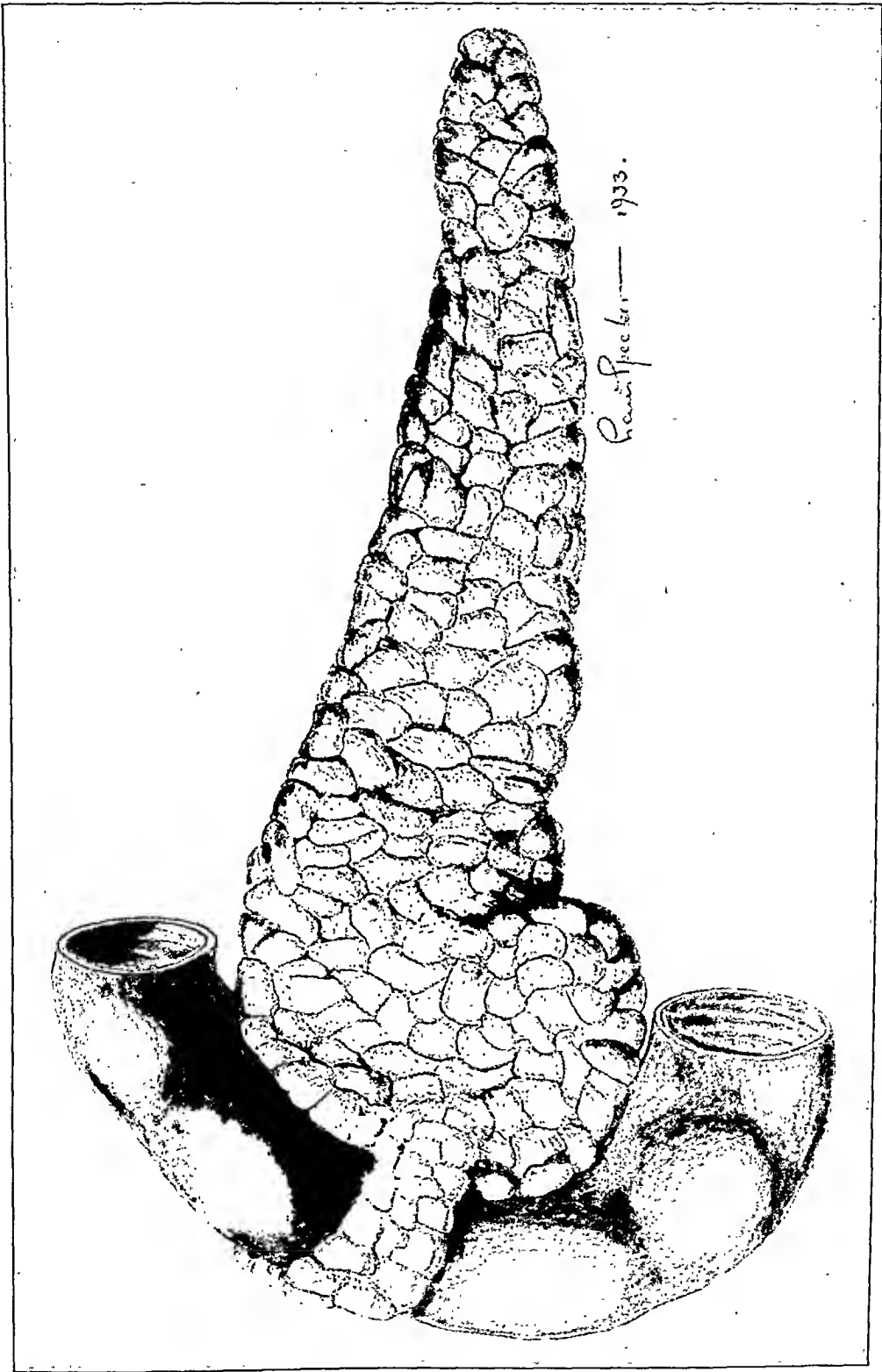


PLATE 25

FIG. 2. Roentgenogram of a case of annular pancreas after injection of bismuth cream through cannula C.

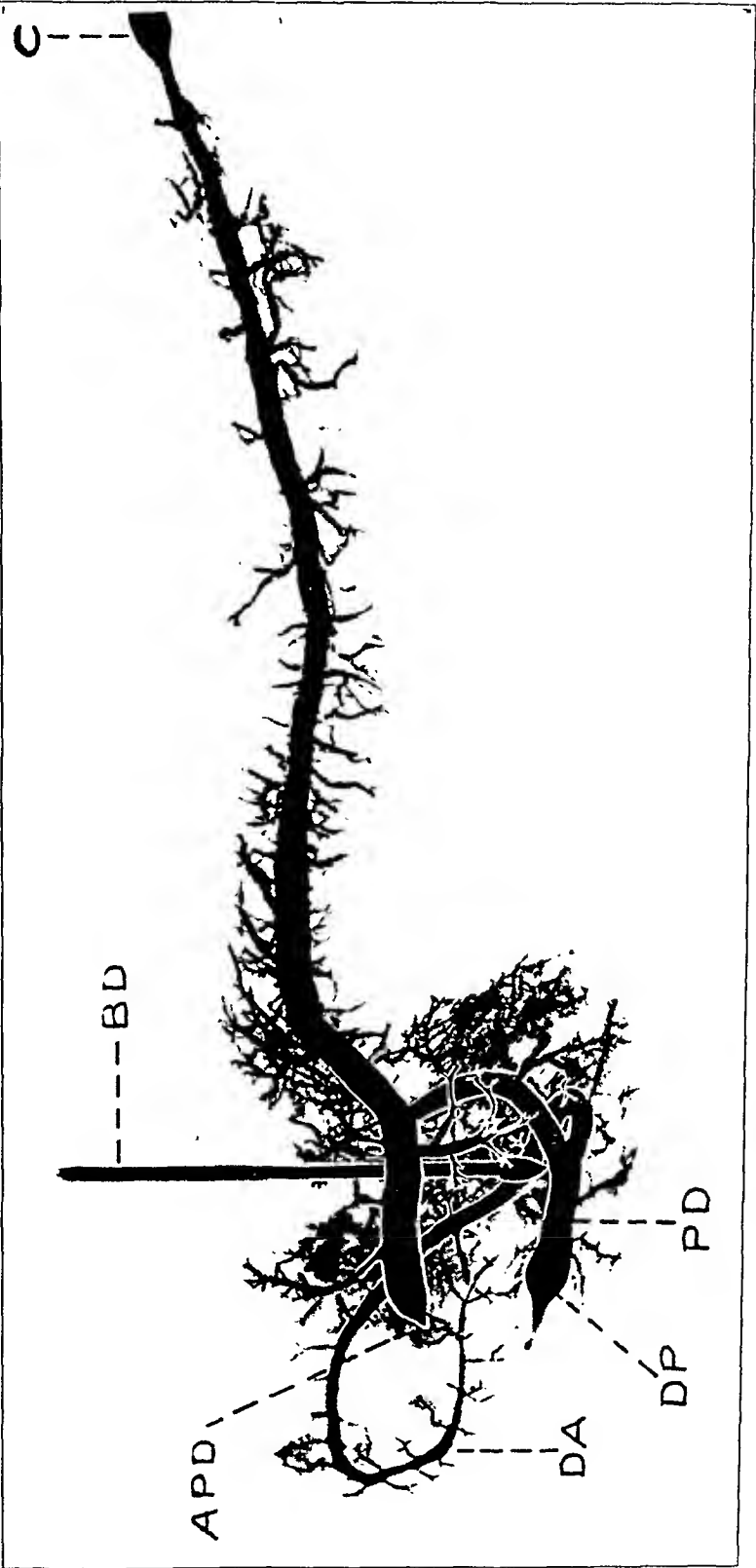
DP = duodenal papilla (ampulla of Vater)

PD = pancreatic duct of Wirsung

APD = accessory pancreatic duct of Santorini

DA = duct of the annular portion

BD = metal probe in the upper portion of the bile duct



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THE AMERICAN JOURNAL OF PATHOLOGY

VOLUME XI

MARCH, 1935

NUMBER 2

HEMORRHAGIC ENCEPHALITIS *

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During the past 15 years we have encountered at autopsy a peculiar involvement of the nervous system. Clinically the patients present a typical picture of encephalitis; pathologically the most striking lesion is a hemorrhagic involvement of the brain. We have called this disease hemorrhagic encephalitis. Because it is so uncommon, a careful review of our cases seems warranted in an attempt to establish certain important clinical and pathological features, and to elicit whatever etiological factors these studies may suggest.

In reviewing the literature on the subject of hemorrhagic encephalitis one is immediately impressed by the great number of reports in which a hemorrhagic condition of the brain followed some infectious, toxic or chemical agent. Such reports have been published by Jacobäus, Schmidt, Abt, Alpers, Winkelman and others. In this study we are concerned, however, with a hemorrhagic disease of the brain which, so far as we can determine, is primary and is not associated with any other pathological condition. Many cases suggesting in their clinical picture encephalitis and showing blood-tinged spinal fluid have been reported as hemorrhagic encephalitis, but most of these patients recovered. This fact renders these reports questionable since the clinical picture is so variable that a postmortem examination is usually necessary to establish the correct diagnosis.

The first cases described which seem to fit the syndrome we are calling hemorrhagic encephalitis were those of Wernicke, published

* Acknowledgment is hereby made of assistance received from the Federal Aid Students.

Received for publication September 17, 1934.

in 1881. He reported 3 cases, all of which terminated fatally and which showed at autopsy numerous petechial hemorrhages in the brain substance. In 1885 Strümpell reported 2 cases which clinically resembled encephalitis and which at autopsy showed markedly distended capillaries, hemorrhages in the white matter and some glial proliferation. In neither were the ganglion cells involved. Strümpell considered his cases as examples of a primary, acute hemorrhagic, non-suppurative encephalitis, a term that very aptly describes the malady. Stäussler in 1902 also reported 2 cases of hemorrhagic encephalitis which he considered toxic or infectious in origin, although he was unable to specify the exact cause in either case. His description of the cases, *viz.*, the acute onset in previously healthy individuals and the presence of numerous small petechiae throughout the brain at postmortem, correspond entirely to the condition that we wish to describe. Unfortunately Stäussler did not give a detailed pathological description of the nervous system in his cases.

More recently Grinker and Stone reported a series of cases of non-suppurative encephalitis. Only one of these falls into the category of what we should call hemorrhagic encephalitis. McIntyre reported 20 cases of an unusual encephalopathy, 13 of which had blood in the spinal fluid. Most of these patients recovered, but the few who died did not show any of the brain changes so characteristic of hemorrhagic encephalitis. Therefore, the author was probably dealing with some other condition and not the one under discussion.

GENERAL FEATURES

Our cases of hemorrhagic encephalitis have appeared sporadically during the past 15 years at the rate of 1 to 2 yearly. The condition is probably much more frequent than the number of our cases would lead us to believe, since our acquaintance with it is limited to those cases that are accidentally discovered at autopsy. In only 1 of these was a correct diagnosis made before death. It seems quite likely that numerous fatal cases of hemorrhagic encephalitis occur yearly but are not examined and therefore go undiagnosed. Probably many mild cases also occur. These are treated as one of the more common and better known ailments, and the individuals recover after an illness of varying length. The lack of severe pathological lesions in some of our cases suggests the possibility that many non-fatal lesions

of this hemorrhagic type occur. In no instance have multiple cases of this illness been reported from one family.

A careful review of our histories reveals no significant etiological factors. A few of the patients gave histories of a previous upper respiratory infection, but even these had completely recovered before the onset of the final illness. Diminished resistance due to physical hardship, serious illness, poor living conditions, malnutrition and overwork seems to play no part in the pathogenesis of this disease, since the patients were, with the exception of one child, well nourished and healthy before the onset of illness.

The majority of the cases occurred in patients under the age of 25 years, the youngest was aged 7 months, the oldest 55 years. Younger age groups are evidently predisposed, but that is all one may conclude. The cases were divided about evenly between the sexes.

The lesion in the brain is chiefly hemorrhagic and predominantly in the white matter. The lesions vary widely in number and size. Undoubtedly this variability accounts for the lack of uniformity in the clinical features of the disease. It is this lack of a definite symptomatology that makes the differentiation from other diseases of the central nervous system so difficult. We must recognize the fact that a description of the clinical features of this illness must be comprehensive enough to include many variations. But in spite of these numerous variations, the disease shows a predominance of a certain combination of symptoms and presents a fairly definite entity with its typical onset, course, morbid anatomy and localization in the brain.

SYMPTOMATOLOGY

The prodromata generally make an abrupt appearance in a previously healthy person. These consist chiefly of headache, lassitude, a slight elevation of temperature, and occasionally of nausea and vomiting. These are followed rapidly by graver symptoms. In the extremely acute case the patient soon lapses into unconsciousness, the temperature rises to alarming heights and death occurs within a few hours after the first onset of the illness. Five of our patients were found unconscious. In these no clinical history was available.

In the less acute cases, those where the patient survives a few days to a few weeks, the early prodromal symptoms (headache, lassitude, elevated temperature) are succeeded by a clouding of the sen-

sorium, the patient becomes dazed and confused, and soon lapses into coma. Convulsions of some type are almost always present, but signs of meningeal irritation, such as slight stiffness of the neck, are observed only occasionally.

Pyrexia must be regarded as a nearly constant symptom, since it was present in almost all the cases and usually remained high throughout the course of the illness (Table I). Occasionally a fluctuating type of fever curve is recorded, but this is the exception rather than the rule. The rise in temperature is usually sudden, reaching heights of 106°–108° F. in a few hours after the onset of illness. No very evident relation exists between the degree of the pyrexia and the course of the disease, since some patients with only moderate fever died within a few hours of onset while others with very high temperatures lived for many days.

Convulsions of some kind were recorded as occurring in 12 cases (Table I). These manifested themselves shortly after the onset of illness; some were tonic in character and continuous until death, some were tonic but periodic in occurrence, some epileptiform in nature, others were generalized and clonic in type, while still others were described as “vermicular movements of the upper and lower extremities.” As a rule, however, convulsions were generalized and, therefore, of very little localizing significance.

Ocular palsies such as ptosis were not noted, while ocular disturbances were recorded in 4 cases. These varied from very slight changes, such as sluggishness of pupillary reactions in 2 cases, to marked mydriasis in the other 2. In no case was mention made of nystagmus or of the presence of an Argyll Robertson sign. Changes in the eyegrounds were rare. However, a fundus examination was recorded in but half the cases. The changes, when they did occur, consisted merely of a slight blurring of the disk. The involvement of other cranial nerves was rare. In one case (No. 14) there were disturbances in swallowing.

Spasticity of the extremities and reflex disturbances were frequent, although their absence was by no means significant (Table I). With the exception of those extremely acute cases where death took place before careful examination could be made, spasticity of the limbs was a constant finding. The spasticity was either periodic or constant, often persisting throughout the illness. The deep reflexes were described as increased, decreased or unequal. Pathological

reflexes were noted in over one-half the cases in which the reflexes were studied. A striking feature regarding them was their varia-

TABLE I
Clinical Features of Hemorrhagic Encephalitis

Case No.	Temperature	Headache	Convulsions	Altered reflexes	Ocular disorder	Meningeal irritation	Miscellaneous	Found unconscious
1	F.		+					+
2			+					+
3								+
4								+
5								+
6			+					
7	104°		-	-	-	-	Nausea, abdominal pain	
8	101°-106°	+	-	+	+	+	Sweating	
9	91.1°	+	-	-	-	-		
10	high	-	+	-	-	-		
11	101°-106.5°	+	+	?	-	-	Sweating	
12	102°-108°	+	-	+	-	-		
13	?	-	+	?	-	+		
14	104°	+	-	-	-	+	Difficulty in swallowing	
15	105°	+	+	+	+	-	Chills	
16	100°-107°	+	+	+	+	-	Malaise	
17	high	?	+	?	?	-	Stupor	
18	98°-104.2	+	+	+	-	-	Stupor	
19	106-106.8°	+	+	+	-	-	Vomiting, chills	
20	104°-107°		+	-	+	+		

bility from day to day or even from hour to hour. Indeed this rapid change of signs and symptoms was common in our cases.

Certain of the patients gave histories of disturbances of sweat secretion, as well as chills of an indefinite nature, diarrhea and

abdominal pain (Cases 7, 8, 11, 15, 19). Aside from the symptoms referable to the nervous system, the somatic findings were negligible. The heart rate was occasionally increased, but no other significant findings are recorded. The clinical features of our 20 cases of hemorrhagic encephalitis are tabulated in Table I.

To recapitulate, hemorrhagic encephalitis may be characterized as an acute ailment of the central nervous system occurring in previously healthy young individuals and manifesting itself by a sudden onset, headache, an abrupt rise of temperature and a rapid loss of consciousness. Convulsions are common, the extremities are spastic, and the reflexes are frequently abnormal and variable. Death ensues in from a few hours to several days after the onset of illness.

LABORATORY FINDINGS

Blood studies were made in only 8 cases (Table II). The changes in the blood were not pathognomonic. There was usually a moderate leukocytosis, the counts rarely exceeding 25,000 leukocytes per cmm., while in 2 cases the leukocyte count was normal. The white count, like the temperature, gave us no indication as to the prognosis of the disease. In all but 1 case the red blood count was within normal limits. The Wassermann reaction was negative in all the cases.

The cerebrospinal fluid was studied in 12 cases and although not always decisive, often provided the most valuable clue to the clinical diagnosis of the disease. Increased pressure was noted in 5 cases (Table II). The cell count in the spinal fluid varied greatly — from none to a large number. Five cases showed no pleocytosis but in the others the cells varied from 10, 35 and 50 cells per cmm. to as high as 560 cells per cmm. in 1 case (Case 20). In many of the fluids polymorphonuclear leukocytes were numerous, often constituting as many as half the cells.

The spinal fluid was generally clear but in 4 cases it was slightly blood-tinged (Table II). It is this latter finding that should make one suspect this ailment. Whenever a bloody spinal fluid is obtained, together with the above clinical picture, hemorrhagic encephalitis must be considered in the differential diagnosis. In only an occasional case, however, do the cerebral hemorrhages approach close enough to the surface of the brain or ventricles to give a blood-tinged fluid.

TABLE II
Laboratory Findings in Hemorrhagic Encephalitis

Case No.	White blood count	Red blood count	Spinal fluid	
			pressure	cells
3	<i>per cmm.</i>	<i>per cmm.</i>		<i>per cmm.</i> Bloody
6			Clear Normal pressure	3 cells
7			Clear Normal pressure	5 cells
8	9,500 (83 % P.M.N.)	5,136,000	Clear Increased pressure	10 cells
9	16,300 (82 % P.M.N.)			
10	8,000	4,150,000	Clear Increased pressure	35 cells
11				Bloody
12			Clear Normal pressure	2 cells
15	17,000		Clear Increased pressure	50 cells
16	14,000 (91 % P.M.N.)	4,600,000	Clear Normal pressure	Bloody
18	11,600 (85 % P.M.N.)		Increased pressure	Bloody
19	21,100 (81 % P.M.N.)		Clear Normal pressure	10 cells
20	25,000 (71 % P.M.N.)	2,160,000	Increased pressure	560 cells

The spinal fluid Wassermann reaction was invariably negative. The urine examinations did not reveal any pathological changes.

CLINICAL DIFFERENTIAL DIAGNOSIS

Although most of our experience has been with the very acute and rapidly fatal form of the disease, it is likely that a considerable number of cases may take a milder form and recover. Numerous transitory cerebral disturbances may be due to this masked form of the disease. Unfortunately, the absence of a blood-tinged spinal fluid

precludes a clinical diagnosis of hemorrhagic encephalitis, even in the extremely severe cases. However, we feel certain that since we meet about 2 cases yearly in our autopsy service, the condition must be more prevalent than this number would seem to indicate and that many cases must escape our attention either because of recovery or misdiagnosis.

It is because of the difficulty of diagnosis that we are unable to make even a tentative statement regarding the prognosis or the sequelae of this disease. A statement of the mortality would be justified only if we knew some method of detecting the milder cases.

The differential diagnosis in hemorrhagic encephalitis must be discussed from two points of view, namely the clinical and the pathological. The latter will be treated in detail in a later paragraph. The scope of this paper does not permit a complete discussion of each of the numerous clinical conditions with which this disease might be confused. We must content ourselves here with merely listing them, together with some of their most complete references.

Clinically the disease that resembles our cases most closely is the lethargic encephalitis of von Economo. Especially is this true of the somnolent form which bears a striking similarity to our cases. Among the numerous other conditions which resemble hemorrhagic encephalitis clinically are: the bulbar type of poliomyelitis; the encephalitis following smallpox and rabies vaccination (Gordon and Rhea, Wilson and Ford, Flexner, Denson, Scott, Hekman, Huber); the encephalitides following the infectious diseases, measles (Lowenburg and Schaller, Skoog, Guthrie, Smith, Box, Fox, Fairbanks, Beach, Jenkins, Bruce, Ford, Horwitt, Walthard), scarlet fever (Boenheim, Neurath, Schilder), typhus (Jarisch, Feldmann, Grodzki, Morawetz), whooping cough (Askin and Zimmerman, Bertoye, Mikulowski, Boenheim), and dysentery (Lenhartz, Buttenwieser); the encephalitides resulting from the various metallic poisonings such as lead (Hassin, Kato, Lewin and Treu, Waterfield, Barron and Habein), and arsenic (Fischer, Hahn, Globus and Ginsburg); the acute form of disseminated sclerosis; the cerebral involvement following botulism (Wilbur and Ophüls, Meyer, Cox, Smith), and alcoholism (Gamper, Ohkuma, Neubürger, Bender and Schilder); cerebral syphilis; brain tumors; meningitides, chiefly tuberculous; influenza (Mouriquand, Bernheim and Boucomont, Eiwin and Wurman, Elmer and Boylan); certain cases of subacute bacterial

endocarditis; and finally cases of spontaneous subarachnoid hemorrhage. The last two conditions deserve particular mention because they may show, besides their confusing clinical history, a blood-tinged spinal fluid, which is the one essential feature in the clinical diagnosis of hemorrhagic encephalitis. In some cases of subacute bacterial endocarditis small emboli lodge in the brain, producing areas of infarction. If these cerebral infarcts are situated close to the surface of the brain or the ventricles, a bloody spinal fluid might be present. We have studied one such case that clinically presented a typical picture of hemorrhagic encephalitis, including the bloody spinal fluid. At autopsy the correct diagnosis was readily apparent. Spontaneous subarachnoid hemorrhage occurs most frequently in young people, and if the bleeding is slow, the numerous clinical findings, together with the bloody spinal fluid, make a diagnosis very difficult. Forbus has studied this subject and has shown that in young people the bleeding is most often due to rupture of an aneurysm of a superficial cerebral artery.

PATHOLOGICAL ALTERATIONS

The lesions found at postmortem in hemorrhagic encephalitis constitute the essential characteristic feature of this disease. Unfortunately, though the lesions are fairly characteristic, they are not pathognomonic. Numerous other conditions (which will be discussed later) may produce a somewhat similar anatomical picture. However, with a good history at hand one should be able, in the presence of the pathological features to be described below, to arrive at a correct diagnosis of this disease. Strümpell, in 1885, was the first to describe the fundamental features of the cerebral involvement in hemorrhagic encephalitis. He considered his cases characteristic enough to constitute a pathological entity, which he described as a "primary, acute, hemorrhagic, non-suppurative encephalitis."

The dura mater shows no change, except for occasional congestion of its vessels. The leptomeninges are also normal, except in a few cases in which they show moderate hyperemia and diffuse edema. There is no thickening or purulent involvement of these membranes. The cerebrospinal fluid pressure may be increased, but the convolutions of the brain do not appear flattened. In 2 of our cases there was

a diffuse thin layer of blood beneath the arachnoid. These extravasations were quite marked, covering a large surface of the cortex and producing a bloody spinal fluid. Careful examination of the brain surfaces revealed no definite bleeding point. Two other cases (Nos. 18 and 20) showed on the surface of the cortex large areas of discoloration and softening, which on section proved to be large hemorrhagic areas that had destroyed the brain tissue and extended from the white substance into the cortex. In only 1 of these cases had the erythrocytes actually broken into the subarachnoid space to produce a blood-tinged spinal fluid (No. 18).

Generally, therefore, (16 of our 20 cases) the external gross appearance of the brain is normal, although occasionally the hemorrhagic process is obvious, even from the external examination.

Coronal sections through the brain reveal a uniform and striking gross picture. Scattered irregularly through the brain, but without localization to a particular region, are numerous sharply circumscribed hemorrhages of variable size and number. These are characteristically located in the white substance, with only an occasional petechia appearing in the cortex (Fig. 1). In many cases the hemorrhages are very small, few in number and either scattered diffusely throughout the tissue or collected in tiny groups in certain regions of the brain, particularly around the ventricles. In other cases these tiny hemorrhages are extremely numerous, completely filling the tissue and producing a speckled appearance in the gross section. These hemorrhages are often so small that it is difficult to determine grossly whether they are true bleeding points or merely dilated vessels. Upon microscopic examination under moderate magnification the true nature of the tiny extravasations can readily be perceived.

In some sections larger, widely scattered, irregular areas are encountered which have the appearance of confluent punctate hemorrhages. These are often surrounded by numerous petechiae of smaller size. The larger hemorrhages are found only in sections where the smaller ones are numerous and are closely packed together, suggesting that these larger hemorrhages are formed by the fusion of the smaller ones. The brain tissue does not appear softened or altered about any of these medium sized hemorrhages.

In an occasional case very large hemorrhages are found (2 of our 20 cases) (Fig. 2). These may be so extensive that they destroy large areas of brain tissue. They not infrequently extend through the

cortex into the subarachnoid space or into the ventricles. These are the solid masses of blood that are visible as discolored areas upon external examination of the brain. Around these massive hemorrhages are seen many minute to moderate sized effusions of blood. Again one gains the impression that the larger extravasations are formed by the fusion of numerous smaller ones. The brain tissue surrounding these large hemorrhages is softened and necrotic.

The large vessels at the base of the brain and their branches are normal. There is no evidence of any sclerosis, thrombosis, or embolism. In none of the cases was there found an aneurysm of these vessels.

The great variation in degree of the hemorrhagic cerebral involvement in this condition can be illustrated by briefly contrasting the gross appearance of the brains in 2 of our cases (Nos. 15 and 20). In the first of these the hemorrhages were almost entirely small and discrete (Fig. 1), while in the second they were massive and accompanied by a large amount of injury to the brain tissue (Fig. 2).

Case 15 was that of a 55 year old female who died after an illness of but a few days duration. At autopsy the meninges appeared normal. Aside from slight distention of the small vessels, no sign of any pathological disturbance was apparent from the external examination of the brain. Serial sections, however, revealed a very extensive hemorrhagic involvement (Fig. 1). Throughout the brain substance there were numerous, small, petechial hemorrhages which, although variable in size and number, always remained comparatively small. These hemorrhages were chiefly disseminated throughout the white substance. There were a few small groups in the subcortical region of the parietal lobes. These at no time fused to form large hemorrhages. Only a few scattered points of bleeding were found in the brain stem and none at all in the cerebellum. The brain tissue did not seem softened, even in the region of the most numerous hemorrhages. The ventricles were clear and their walls contained no accumulation of red cells. The findings in this case illustrate the small, uniform, punctate type of lesion so commonly seen in hemorrhagic encephalitis of the rapidly fatal type. These hemorrhages are occasionally so small (No. 19) that grossly one is unable to determine whether they are true extravasations of blood or merely dilated vessels.

In contrast to this case, No. 20 exemplifies those with massive

irregular hemorrhages and with a marked destruction of brain tissue (Fig. 2). This patient, a child, died 2 weeks after the onset of illness. On external examination of the brain, the pathological involvement was immediately apparent. On the inferior surface of the left frontal lobe and on the lateral surfaces of the left parietal and temporal lobes of the brain there appeared a purplish discoloration and a marked softening of the cortex. The right half of the cerebrum, the pons, the medulla and the cerebellum seemed normal externally. Sections revealed a marked involvement of the left half of the brain and a much milder process on the right.

Sections through the left cerebral hemisphere revealed large hemorrhages containing fresh and old blood, and a marked destruction and softening of the brain tissue. The hemorrhages varied in size from pin-point extravasations to confluent areas measuring 7 to 9 cm. in diameter and containing masses of old clotted blood. A large area of involvement was present in the anterior portion of the left frontal lobe where the necrotic brain substance extended almost to the cortex. In the left parietal and temporal regions the hemorrhage involved large areas of the white substance and extended through the cortex to the surface of the brain, but did not break through into the subarachnoid space. The left occipital lobe contained only an occasional small hemorrhage. In the upper part of this region there was a single hemorrhage measuring 1 cm. in each dimension. The ventricles were free from blood, although numerous petechiae were scattered throughout their walls. The basal nuclei were strikingly free from involvement.

The right half of the brain contained numerous, small petechial hemorrhages scattered through the white substance, the internal capsule, and the anterior extremities of the caudate and of the lenticular nuclei. This latter region appeared somewhat softened. The right lateral ventricle was free from blood, although the tissue surrounding its anterior horn was hemorrhagic and necrotic. This area was the only one in the right half of the brain in which the hemorrhages assumed a notable size, surpassing in magnitude the lesions described in the previous case.

The lesions described in the left half of this brain present a striking contrast to those in No. 15. Our series of cases presented all gradations between the extremes described above, as well as all possible degrees of dissemination and types of localization of the lesions.

MICROSCOPIC ALTERATIONS

Normal Brain

To appreciate fully the microscopic changes appearing in the central nervous system in hemorrhagic encephalitis, it is necessary to have a thorough understanding of the normal histology of the cerebral vessels, as well as the normal variations that may be encountered in a routine study of apparently normal brains within any single age group. In order to study the normal brain we selected from our coroner's service thirty apparently normal brains from individuals under 35 years of age, all of whom died from some extra-cerebral condition such as gun-shot wounds of the abdomen or various other abdominal conditions. Brains of younger people were used in order to eliminate the problem of normal age changes, and to facilitate a comparison with our cases of hemorrhagic encephalitis which usually occurred in younger individuals.

The arteries that supply the brain are quite different from similar sized arteries in other parts of the body. The average small cerebral artery is characterized by a well developed elastica interna, and a media composed of a large amount of collagenous tissue with a few elastic fibrils and muscle fibers. The adventitial coat is scanty or entirely absent.

The internal elastic lamina is the most conspicuous part of the cerebral vessel and is both relatively and absolutely thicker than in similar sized vessels elsewhere. Often fine elastic fibrils can be observed extending outward from the elastica interna and branching through the medial layer.

The media consists of concentrically arranged, anastomosing collagenous fibers, among which are found some smooth muscle tissue and a few elastic strands. With the azocarmine stain it is apparent that even in young individuals the collagenous tissue is relatively much more abundant in the cerebral arteries than in arteries of corresponding size elsewhere.

The adventitia is often entirely absent in the small cerebral arteries. When it is present it consists of a few strands of collagenous fibers which partially or completely surround the medial layer.

The arteries of the white substance of the brain differ somewhat from those of the cortex in that they contain even less muscle in their media.

A more complete description of the cerebral vessels will appear in a separate publication.

In a survey of a series of normal brains there were found about the vessels a few changes which occur often enough to indicate that they are apparently not of pathological significance. Most of the capillaries and tiny arterioles are empty and collapsed, or at most contain but few erythrocytes. The larger arterioles and the small arteries are often filled with red cells. In some normal brains, however, many of the capillaries are distended, and in still others all of the cerebral vessels from the smallest to the largest are full of erythrocytes. Occasionally a small vessel is observed about which a very thin collar of erythrocytes can be seen. This perivascular hemorrhage, if severe, is a definite lesion, but it may be detected occasionally about vessels in normal brains. These extravasations are always very small and consist of only a few cells which partially surround the vessel. A few arteries are observed which present a slight increase of mononuclear cells in and about their outer walls. From these observations it becomes apparent that a moderate capillary dilatation, an occasional perivascular hemorrhage, or slight mononuclear infiltration, must be considered in the range of normal variation.

Our routine microscopic studies of the nervous system included sections taken from numerous cortical areas, the basal ganglia, the midbrain, the cerebellum and the medulla oblongata. These were stained in one or more of the following ways: hematoxylin-eosin, Weigert's myelin sheath stain, iron hematoxylin-Van Gieson, Nissl's method, Bielschowsky's stain, Sudan III stain for fat, Cajal's gold sublimate stain, and del Río-Hortega's silver carbonate method. The last two methods proved the most successful in our hands in demonstrating the detailed structure of the astrocytes and microglia. In both of these methods the fresh tissue was fixed in Cajal's formalin-ammonium-bromide solution; the tissue that had already been fixed in formalin was subjected to bromuration. This latter process was suggested by Globus. It consists in placing formalin-fixed tissue in distilled water to which has been added one drop of strong ammonium hydroxide for each cc. of water. The tissue is left in this solution overnight, then washed and placed in a 10 per cent hydrobromic acid solution for 1 to 3 hours. Sections are then ready for impregnation with either gold or silver.

Appearance of Brain in Hemorrhagic Encephalitis

The most characteristic feature of the microscopic appearance of the brain in hemorrhagic encephalitis is the presence of numerous hemorrhages that vary in magnitude from extensive extravasations which destroy much brain tissue to tiny perivascular bleedings. All degrees of variation can be found between these two extremes. Usually the hemorrhages are scattered irregularly throughout the white substance of the cerebrum, occasionally some are grouped together in certain regions, while the rest of the tissue contains only dilated capillaries or a few perivascular erythrocytes. The hemorrhages, unless very extensive, are strictly limited to the white substance. When they are large they may tear through the cortex and reach the surface of the brain (Fig. 3). These massive hemorrhages are usually surrounded by petechiae of variable size. It is often possible to reconstruct the successive stages in the fusion of these petechiae to form the larger hemorrhagic areas. In many cases what appears grossly to be solid masses of extravasated blood is readily recognized microscopically to consist of a conglomeration of numerous small hemorrhages.

Most of the small hemorrhages assume a perivascular arrangement (Fig. 4). The earliest stage in their formation consists of a few erythrocytes scattered about the tiny vessels. When the larger arteries are involved the red cells at first fill the perivascular lymph space, tearing the glial fibers away from the vessel wall. As the hemorrhages increase in size, they break through the ring of glial tissue into the surrounding tissue, causing various degrees of injury to the brain and the interruption of some of the nerve fibers in the vicinity. Sections taken shortly after this tissue injury has occurred show little or no reaction of the cerebral elements, but after a few days extensive alterations are visible. These changes will be discussed in a later paragraph.

Ball of Solid Hemorrhages: Bleeding from the larger vessels always results in solid accumulations of red cells termed "ball hemorrhages." These, either singly or fused, are by far the most frequent type of hemorrhage observed in the brain (Fig. 5). Although they often are so numerous that they replace large areas of brain tissue, they have a tendency to remain discrete. They usually have at their center a ruptured vessel (Fig. 4). In many, the vessel is not visible,

although the rounded uniform arrangement of the hemorrhage certainly suggests a vascular origin. The vascular injury that permits these solid hemorrhages to be formed can be demonstrated by carefully studying the walls of the involved vessels. In the early cases little or no change can be detected in spite of a most careful study. Evidently sufficient time has not elapsed for the morphological changes to occur. One of the earliest changes apparent in the vessels is a loss of the normal staining reactions in certain portions, suggesting an early degeneration. This is followed by a thinning out of certain areas of the elastic lamina with a resultant defect of the wall. In the smaller vessels the collagenous layer of the wall is too thin to provide a sufficient support in those regions where the elastica has degenerated, and soon one may detect a bulging of the weakened wall and a streaming of red cells through into the surrounding tissue. The larger arteries have a thicker supporting collagenous membrane and a few muscle fibers which prevent bleeding even after the elastica interna has thinned out and ruptured. However, in some of these arteries, after the elastica has degenerated, the collagenous fibers of the media also become frayed and torn in various areas. The erythrocytes then follow the tears in the wall and eventually break through to the outside. Many vessels present a swelling and hyaline degeneration of the elements of their walls. There is a loss of differentiation into the normal layers and the walls become homogeneous and rupture.

Still another change is frequently observed, especially about the smaller vessels. This consists in endothelial proliferation which may be moderate, replacing the vessel wall, or so extensive as to reach tumor-like dimensions. No hemorrhages are observed about these vessels, suggesting that the endothelial proliferation has probably strengthened the walls sufficiently to prevent rupture and hemorrhage.

Ring Hemorrhages: Another type of hemorrhage which often occurs is the so-called ring hemorrhage. This presents a clear uninvolved center surrounded by a dense ring of erythrocytes (Figs. 6, 7). It is caused by the rupture of numerous tiny capillaries which surround the larger vessels. They, therefore, take the form of a partial or complete ring, depending upon the degree of capillary disruption. When the capillary damage is very severe the red blood cells infiltrate into the clear center of the ring, producing the appearance of a

solid hemorrhage, with the exception that the hemorrhage in these cases is usually less dense in the center than in the periphery. Often the clear centers of these ring bleedings consist of degenerated brain tissue infiltrated with numerous scavenger cells. The damage to the brain tissue surrounding these hemorrhages is similar to that in the solid type and, as in those, depends upon the size of the extravasation.

Diffuse Hemorrhages: The cerebral hemorrhages are not necessarily limited to the region of the blood vessels but often appear as diffuse extravasations (Fig. 8). When only a few erythrocytes are found scattered through the nervous tissue there seems to be no definite injury to the immediate structures. As these diffuse hemorrhages become more extensive the damage to the brain tissue increases. In the small hemorrhages the red cells merely push the cerebral fibers apart without causing them to rupture, but the increase in the number of erythrocytes results finally in a rupture of many of the fibers, followed by demyelination and infiltration of fat granule cells. This latter picture can be very readily demonstrated with a modified silver stain (Fig. 9).

Perivascular Demyelination: When death occurs in hemorrhagic encephalitis within the first 24 hours of the disease, hemorrhages alone are detectable in the brain substance. Undoubtedly some damage has occurred, but insufficient time has elapsed for evidence of that injury to become visible microscopically. Many patients survive the first few days, and in these it is easy to observe the characteristic alterations in the brain tissue which may not only accompany the hemorrhages, but may also occur independently of them. The latter changes usually occur about the blood vessels, producing a perivascular demyelination (Fig. 10). The perivascular alterations are at first very slight, consisting of myelin destruction with a beginning infiltration of scavenger cells (Fig. 11). In the more advanced stages the perivascular demyelination is extensive, and the infiltration of scavenger cells filled with fatty debris is marked. A slight proliferative reaction of the neuroglia situated near the periphery of the destroyed area may now be detected. The end result of this destructive process is a complete demyelination around the vessel (Fig. 10). The destroyed area ultimately contains only a few scavenger cells; but there is a great increase in glial fibers laid down by the proliferating astrocytes. There are no hemorrhages about

these vessels. These areas of non-hemorrhagic perivascular demyelination strongly suggest the possibility that in this disease some neurotoxin is disseminated by means of the cerebral vessels and produces either vessel injury and hemorrhage, or no vessel injury but perivascular tissue damage, depending upon its localization.

The cytological changes that occur both in the non-hemorrhagic degenerated areas and in the tissue surrounding large hemorrhages are similar and will, therefore, be described together. The chief cellular elements involved in cerebral injury are the microcytes and the astrocytes. Special stains are essential for a detailed study of these elements. These stains have been listed in a previous paragraph.

Microglial Changes: The normal microglial cells were first studied in detail in 1921 by del Río-Hortega who worked out their morphological and structural details, their transformation into rod cells and fat granule cells, their motility and their phagocytic functions. This author firmly believed that these cells are mesodermal in origin. This view is widely accepted (Penfield, Jiménez de Asúa, Belloni, Ramirez Corria, Russell and others). Numerous names have been applied to this cell, such as Nissl's Stäbchenzellen, ameboid wandering cells, granulo-adipose cells, Gitterzellen, compound granular cells, scavenger cells and fat granule cells. The last two terms will be used interchangeably in this discussion.

The microglial cells invade areas of cerebral damage and remove the destroyed tissue. In order to discharge their functions these cells develop marked migratory and phagocytic properties. Shortly after a brain injury occurs, regardless of the nature of the injurious agent, these cells draw in their processes and move toward the injured area. After reaching the site of injury they may increase in size and number (Fig. 12). Some investigators still doubt their migratory ability, but sufficient evidence is available to establish their migratory and phagocytic powers. The tendency of these fat-laden scavenger cells to accumulate about the blood vessels and even to invade their lumens speaks strongly for their migratory faculty.

The normal resting microglial cells can usually be identified with the routine nuclear stains, since their nuclei are the smallest in the nervous framework and are variable in shape, being round, oval or triangular. They contain a large number of deeply staining chromatin granules and have a distinct nuclear membrane. Their cytoplasm is not visible with ordinary stains. Special staining reveals a scanty

cytoplasm which is prolonged into numerous wavy and branched processes that gradually thin out and terminate some distance from the cell body. Normal microglial cells may be unipolar, bipolar, or multipolar; the latter are the most frequent (Fig. 12).

In the early destructive changes in hemorrhagic encephalitis the increase in the number of microglial cells is slight. Most of these cells are elongated to form the so called "rod cells." The transitions between these rod forms and the normal microglia can readily be demonstrated in a study of the invading cells. As del Río-Hortega and Penfield have pointed out, there is a gradual retraction of the cell processes, resulting in the formation of cells with elongated nuclei and two broad bands of perinuclear cytoplasm. These cells have a typical rod shape. Only an occasional cell is seen that has developed into the rounded fat granule form. When fat stains are applied in this early stage only a few of the altered forms are found to contain fat granules, since the rod cells do not possess marked phagocytic ability. If the patient succumbs early in the disease, this is as far as the morphological changes have advanced.

The older degenerated lesions are filled with typical scavenger cells. As one passes from the normal tissue into the injured areas the complete metamorphosis of the microglia into rod cells and finally into fat granule cells is readily observed. The latter cells are globular in form, have no cytoplasmic processes and vary widely in size and shape (Fig. 11). They are usually filled with fat droplets. They are very abundant in the regions of severe degeneration and represent an advanced stage in the process of the phagocytosis and removal of destroyed tissue and extravasated red blood cells. A fat stain applied to material at this period shows all stages in the process of phagocytosis. Some cells contain only a few fat granules; others are completely filled. The scavenger cells vary in size from small cells with a central nucleus and a few fine fat droplets to large, irregular bodies in which the nucleus becomes eccentric or is entirely invisible because of the large accumulations of fat. Many cells reach tremendous dimensions. These are often multinucleated. A perivascular arrangement of these fat-filled cells was not observed in any of the sections. A moderate amount of fat may be observed lying free in the demyelinated areas.

In the periphery of the larger hemorrhages the fat granule cells ingest not only fat but also red cells and parts of red cells. Pecul-

ially, hemorrhages do not stimulate the changes in the microglia in the absence of brain injury. Granule cells were never found around the smaller hemorrhages. Their faculty for ingesting red cells seems to be subordinate to the removal of the fatty débris.

Some scavenger cells undergo degeneration, and present pyknosis of the nuclei and a cytoplasmic disintegration. These cells are infrequent, but quite characteristic when present.

Changes in the Astrocytes: When the microglial cells have accomplished their phagocytic function and have begun to decrease in number, the astrocytes begin to undergo various proliferative changes. The normal resting astrocyte possesses an irregularly oval, pale staining nucleus which contains a few scattered chromatin granules. The cell body is large and radiating from it are numerous branching processes of variable length and thickness. These cells proliferate only in the final stages of the tissue destruction. The neuroglial proliferation is accompanied by a swelling both of the nucleus and of the cell body. The cytoplasm becomes finely granular and the nucleus becomes larger and more irregular, often polymorphous. Accompanying this hypertrophy there is an increase in size and number of the cell fibers, producing a zone of fairly intense gliosis. The astrocytes in the immediate vicinity of the larger hemorrhages undergo degenerative changes due to pressure of the hemorrhagic extravasations. They swell, the cytoplasm becomes coarsely granular and the processes become fragmented and scattered. Their nuclei become smaller and pyknotic. In many of these cells both the nucleus and the processes disappear, leaving only a granular degenerating cell body. The large degenerating astrocytes are scattered among the numerous fat granule cells that have invaded the dead tissue. Occasionally a cell of this type contains a few fine fat droplets, but they are never observed engorged with fat as are the scavenger cells. Apparently normal astrocytes within an area of degeneration never contain fat droplets. The fact that these cells contain such small amounts of fat, in spite of their presence in the center of the necrotic demyelinated area, speaks against their phagocytic ability.

Nerve Cell Changes: Consistent specific changes in the ganglion cells have not been observed in our cases of hemorrhagic encephalitis. This favors the view that alterations in these cells are due to the destructive action of the large hemorrhages which occasionally rup-

ture into the cortex. Scattered through these regions in which the hemorrhage has involved the gray substance are found isolated ganglion cells showing various degrees of degeneration — fragmentation of their processes, shrinkage of the cell body and eccentricity or complete absence of the nucleus. Other cells are large and swollen with a granular vacuolated cytoplasm and a complete absence of the cell processes. All stages in the degeneration of the nerve cells can be observed from slight swelling to complete homogeneity, disintegration and dissolution. With the Nissl stain these cells show partial to complete chromatolysis. There is no neuronophagy. The injured ganglion cells are not replaced by glial cells. The nerve cell changes do not occur simultaneously over large areas of cortical substance, but are found in only a few ganglion cells here and there within the dead brain tissue surrounding the larger hemorrhages.

A search for specific inclusions in an attempt to relate this condition to the virus diseases has been fruitless to date.

Occasionally a blood vessel is observed with slight perivascular infiltration of mononuclears. A few widely scattered polymorphonuclear leukocytes are occasionally observed in the area of degeneration. One of the brains contained a slight increase in polymorphonuclears, but the lesions were predominantly hemorrhagic and necrotic.

No attempt will be made to describe the microscopic findings in the nervous system of each individual case. However, the essential pathological features of each case in which sufficient material was available for study are summarized in Table III.

The internal organs show no characteristic findings. The lack of pulmonary involvement is quite striking, particularly in view of the fact that some of the patients complained of upper respiratory symptoms sometime before the onset of illness. When lung complications are present they are mostly terminal in nature. The other internal organs similarly show no pathological features.

PATHOLOGICAL DIFFERENTIAL DIAGNOSIS

The pathological picture just described is, in itself, hardly diagnostic of hemorrhagic encephalitis since a similar one may occur in numerous other conditions. This is not surprising, since it is recognized that the central nervous system may react in a similar manner to a large variety of irritants. It is, therefore, essential to obtain a detailed history and physical examination and to perform a careful

autopsy in order to elucidate the etiological factors responsible for the hemorrhagic picture. Cerebral hemorrhages have been reported in such diverse conditions as: the encephalitides following measles, scarlet fever, dysentery, typhus; poisoning with arsenic, methyl

TABLE III

Microscopic Alterations in the Brain in Cases of Hemorrhagic Encephalitis

Case No.	Nerve cell injury		Brain tissue injury	Microcyte infiltration	Perivascular demyelination	Petechial hemorrhages	Large hemorrhages	Diffuse hemorrhages	Localized hemorrhages	Tissue injured around hemorrhage	Perivascular hemorrhage	Alterations in vessel wall	Thrombosis of vessels	Infiltration of mononuclears
	Chromatolysis	Other changes												
1.....	-	-	±	-	-	+	+	+	-	+	+	±	-	±
2.....	-	-	-	-	-	+	±	+	-	±	+	-	-	-
3.....	-	-	-	-	-	+	-	+	-	-	+	-	-	-
4.....	-	-	-	-	-	+	-	-	+	-	+	±	-	-
5.....	-	-	-	-	-	+	-	+	-	-	+	±	-	±
7.....	-	-	-	-	-	+	-	+	-	-	+	+	-	-
9.....	-	-	+	±	±	+	±	+	-	-	+	+	-	+
10.....	-	-	+	+	±	+	-	+	-	-	+	+	-	+
11.....	±	±	-	-	-	+	-	+	-	-	+	+	-	±
12.....	-	-	-	-	±	+	-	+	-	-	+	+	-	-
13.....	-	-	-	-	-	+	-	-	+	-	+	±	-	±
14.....	-	-	-	-	-	+	-	+	-	-	+	-	-	-
15.....	+	+	+	+	+	+	+	+	-	+	+	+	-	-
16.....	-	-	-	+	+	+	-	+	+	+	+	+	-	-
17.....	-	-	-	-	±	+	-	+	-	-	+	±	±	±
18.....	±	±	+	+	±	+	+	+	-	+	+	+	-	-
20.....	+	+	+	+	+	+	+	+	-	+	+	+	-	-

chloride, phosphorus and alcohol; trauma to the head without skull fracture; fat embolism after fracture of bones; and hemorrhagic or venous infarction of the brain. We have had the opportunity to study the cerebral lesions in many of these conditions, and a discussion of the findings in these, in order to facilitate a comparison between them and hemorrhagic encephalitis, seems warranted.

Arsenic Poisoning

The cerebral lesions of arsenic encephalitis are often hemorrhagic. It is for this reason that it has been called "hemorrhagic encephalitis" or "purpura hemorrhagica" by investigators such as Pritzi, Pines and Prigonikow, Almkvist, and Scott and Moore. The cerebral hemorrhages are so common in individuals dying of arsenic poisoning that some investigators have been led to believe that all sudden unexplained deaths in which hemorrhages are found in the brain are due to poisoning with this substance. This contention seems to gain strength from the observation that a faintly positive Reinsch, Marsh, or Gutzeit test for arsenic is obtained from the brain tissue of an occasional one of these cases. However, arsenic is so commonly used in industry and is so widely distributed in nature, that it is not surprising that small quantities should constantly enter the human body. The amount of arsenic normally present in adults would naturally vary greatly, depending upon the character of the individual's food, drink, medication or environment. As Myers has shown, this more or less normal ingestion of small amounts of arsenic renders a positive chemical test alone, as a criterion for arsenical death, inconclusive.

Since the introduction of arsenicals in the treatment of syphilis, cerebral accidents known to be caused by arsenic have increased. In 1933 Globus and Ginsburg reviewed the literature on this subject. They found 74 cases with complete autopsy findings, although numerous incomplete cases have been reported (Almkvist, Hahn, Nonne, Pritzi, Mingazzini). A review of 1 case in our service will help clarify the clinical picture and the pathological features of this condition.

CASE 1. A. H., a 21 year old white female, was admitted to the hospital unconscious. She had recently been exposed to syphilis and 11 days before admission she had received 0.6 gm. of neosalvarsan intravenously, followed 6 days later by a similar injection. Two days after this second treatment she suddenly developed a severe headache, lost her appetite and became lethargic. The following day she was more listless and developed generalized convulsions. The family physician who was called obtained an incomplete history. Finding a marked rigidity of the neck he made a tentative diagnosis of meningitis and ordered the patient to the hospital. Upon admission to the hospital she was comatose, her neck was rigid and her blood pressure 130/80. The chest and abdomen appeared normal. Reflexes were normal, but the neurological examination was otherwise unsatisfactory because of the patient's condition. A confluent macular eruption covered her arms and shoulders.

Laboratory Examination: The urine was normal. The white blood count was 13,000 with 68 per cent polymorphonuclears and 31 per cent lymphocytes. The blood sugar was 0.14 per cent, urea nitrogen 30.1 mg. per 100 cc. of blood, creatinin 2 mg. per 100 cc. of blood, and the icterus index was 13. The spinal fluid was normal in appearance and under normal pressure.

The patient's temperature was 102° F. on admission. It rose rapidly to 106° F., where it remained until the time of death, which occurred on the second day of the hospital stay.

Autopsy: External examination of the body revealed mottling of the lateral surface of the right shoulder, the right arm and the left hip, with very tiny, closely-set petechial hemorrhages. Nothing else of note was observed externally. The pleural and pericardial membranes were normal. The heart showed a few, scattered, subepicardial petechiae but was otherwise negative. The lungs were somewhat heavier than normal and showed marked posterior hypostasis and a slight terminal pneumonia. A few tiny hemorrhages were scattered through the bladder wall. The remaining organs, with the exception of the brain, appeared normal.

The scalp, calvarium and meninges were all normal. Coronal sections through the brain revealed numerous small hemorrhages. Bilaterally in the white substance at the lateral inferior margin of the putamen there were softened areas measuring 2.5 by 1.5 cm., surrounded by numerous hemorrhages. In the anterior medial portions of both temporal lobes in the region of the uncus and amygdaloid nuclei, there were other slightly softened areas surrounded by densely packed petechiae. Many hemorrhages were seen on the inferior surface of the corpus callosum, on the superior and lateral walls of the lateral ventricles, and in the posterior portions of the brain.

Microscopic Examination: The most characteristic feature is the presence of numerous hemorrhages scattered throughout the white substance (Fig. 13). Extravasations are chiefly around the capillaries and precapillaries and, therefore, are mostly of the ring type. They vary in size, extending occasionally only into the perivascular spaces, but more often involving the surrounding tissue. A few lymphocytes and polymorphonuclears are found within the hemorrhages. In places the extravasations are so numerous and diffuse that they fuse to form massive hemorrhages. Numerous small hemorrhages are also observed in the cerebral cortex. Many of the vessels are injured, showing endothelial swelling as well as hyalini-

zation of the vessel walls. Numerous vessel walls are infiltrated with polymorphonuclear leukocytes and lymphocytes. The vessel changes occur with or without perivascular hemorrhage. There are frequently found in the vessels hyaline or cellular thrombi that completely fill their lumens (Fig. 13). There is no destruction of brain tissue, either about the non-hemorrhagic injured vessels or at the periphery of the hemorrhages. No reaction is observed on the part of the neuroglia or of the microglia. The large nerve cells are intact. The characteristic findings of syphilis, namely perivascular and diffuse lymphocytic infiltration, as well as endarteritis, are altogether lacking.

Comment: The findings in this case correspond with those reported in the literature. Thrombosis of vessels appears to be a very common occurrence (Pritzi, Wechselmann, Scott and Moore, von Marschalkó, and others). The question of the localization of the hemorrhages in the white substance of the brain is somewhat unsettled. In our case, extravasations were also observed in the cerebral cortex. This is in agreement with the observations of Almkvist and Post, although other investigators have been able to detect them only in the white substance. Nonne and Mingazzini have reported hemorrhagic lesions involving both the gray and white matter of the cord.

How does this pathological picture compare with that of hemorrhagic encephalitis? Although the hemorrhages may be very similar, there are some pathological features that facilitate their differentiation. The presence of hemorrhages in the cord and cerebral cortex, of lymphocytic and polymorphonuclear infiltrations, of numerous thrombosed vessels, and of petechiae in other organs are all foreign to true hemorrhagic encephalitis and should make one suspect some other condition. However, the microscopic variations are usually insufficient to warrant the differentiation, and one must rely upon the history, together with supplementary chemical tests, for the diagnosis of arsphenamine poisoning.

In none of our 20 cases of hemorrhagic encephalitis was the history in the least suggestive of arsenic poisoning. However, because of the resemblance of the cerebral lesions to those in arsenic poisoning, and also because some individuals have claimed that this chemical is the causative agent in cases similar to ours, we tested three brains from our series of true hemorrhagic encephalitis, together with four nor-

mal controls, for the presence of arsenic. One hundred gm. of tissue from each brain was used and tested by the Marsh method. One brain of true hemorrhagic encephalitis showed a faint trace of arsenic, while the rest were all negative. Of the controls, three showed similar traces of the arsenic. All the chemicals used in the test, as well as the formalin, proved negative for arsenic. This, we believe, is adequate proof that the etiological factor in our cases is not arsenic.

Several other substances, such as methyl chloride, phosphorus and alcohol have been reported as producing cerebral hemorrhages, but our personal experience has not included such cases. These are not disturbing clinically, however, since a careful history usually permits a correct diagnosis.

Methyl Chloride Poisoning

Methyl chloride poisoning was first described by Gerbis in 1914. Since then 72 cases with 11 deaths have been reported. In man, cerebral hemorrhages are very uncommon, although they are quite characteristic of the lesions produced in the brains of guinea pigs exposed experimentally to the gas.

Phosphorus Poisoning

Spielmeyer was one of the first to describe cerebral hemorrhages as a result of phosphorus poisoning. Such cases are infrequent. The chemical usually does not produce cerebral symptoms. Wertham, in reviewing the literature on this subject, has pointed out that by far the most frequent cerebral lesion is a destruction of the nerve cells. Vascular injury with bleeding is rare.

Alcohol Poisoning

Cerebral hemorrhages are occasionally found in patients dying of alcoholism, an observation first made by Wernicke. His original paper cited 3 cases, only 1 of which followed alcoholism. Since this publication Wernicke's work has been confirmed by Bender and Schilder, Marchiafava and Kant. It appears that these hemorrhages are by no means constant. When they do occur there is a tendency for them to localize in the cortex.

Infectious Encephalitis

Cerebral symptoms following severe infections are frequent. Since most of the cases recover, only a few rather incomplete autopsy reports are available for study (Mouriquand, Bernheim and Boucomont, Eiwin and Wurman, Parnell and Dudley). Schmidt, as early as 1905, reported a series of cases of cerebral hemorrhages in septic diphtheria and pneumonia, while Grinker and Stone published 5 cases in which cerebral symptoms were apparently due to some toxin in the nasopharynx. These workers believed that any severe upper respiratory infection might be responsible for such cerebral involvement. Numerous isolated reports of cerebral hemorrhage have been recorded following specific infectious diseases such as measles, dysentery, typhus and scarlet fever (Musser and Hauser, Bittenwieser, Herzog). These cerebral hemorrhagic manifestations following infections are uncommon and almost always the history, together with other postmortem findings, is sufficient to point to the correct diagnosis. Two cases of postinfectious cerebral hemorrhage have come under our observation, one following scarlet fever and the other pneumonia. A clinical and pathological description of these cases is given to enable one to contrast them with our cases of true hemorrhagic encephalitis.

CASE 1. A white male, aged 39 years, was admitted to the hospital in a very critical condition. He had been ill for several days. At the time of admission a complete pneumonic involvement of both lungs was found. The patient died a few days later in a septic state.

Autopsy: The lungs were heavy and almost completely consolidated. The other internal organs were essentially normal, with the exception of slight cloudy swelling of the liver and kidneys. The external examination of the brain was negative. Coronal sections revealed numerous discrete petechiae scattered throughout the entire white substance (Fig. 15). There was no tendency to localize in any particular region. The cortex contained but few extravasations. No tissue necrosis was apparent. The ventricles were clear.

Microscopic Examination: There is marked distention of the cerebral arteries. Numerous tiny vessels are dilated and filled with blood. Many ring hemorrhages are scattered through the white substance. These are uniform in size and shape. Accumulations of erythrocytes about the larger vessels, as well as diffuse hemorrhages

independent of vessels, are uncommon. Very little change is detected in the vessel walls, although an occasional vessel is thrombosed. No tissue injury is observed nor is there any cellular reaction visible. The nerve cells are somewhat shrunken but their processes are still intact. This case, because of its clinical features and because of the pathological findings in the lungs, offers but little difficulty in the way of diagnosis.

CASE 2. A white female, aged 32 years, was suddenly taken ill with a sore throat, fever, chills and restlessness. The following day she developed a generalized bright red rash which a private physician diagnosed as scarlet fever. During the next few days she became slightly irrational, restless, noisy and uncontrollable. She was, therefore, sent to the hospital. Past infectious diseases included measles, mumps and whooping cough.

On physical examination, the patient was found to have a temperature of 105.8° F. and a pulse of 132. The blood pressure was not recorded. She was very restless and irrational, and mumbled indistinctly. Her face was flushed, the skin was hot and dry, and there was a generalized erythema of the skin over the trunk and extremities, on which was superimposed a fine, discrete, pin-point eruption about the hair follicles. The pupils were equal and reacted to light. The mouth showed an erythematous mucous membrane; the soft palate and uvula were swollen and red. The remainder of the physical examination was negative.

On admission the patient's temperature was so high and the delirium so marked that it was deemed advisable to give her scarlet fever antitoxin. The following day the rash had almost entirely faded. However, she developed generalized rhonchi throughout both lungs, and soon became cyanotic. Respirations became irregular and she died on the following day.

The blood count showed 83 per cent hemoglobin, 4,610,000 erythrocytes, 16,700 leukocytes, with a differential count of 90 per cent polymorphonuclears and 10 per cent mononuclears.

Autopsy: The external examination revealed no rash but there was a slight, fine desquamation about the cheeks. Aside from the lungs, the internal organs showed nothing of note. The lungs were heavy and almost completely consolidated. On section they were found to be dark red in color and full of pus.

External examination of the head showed the scalp and calvarium to be normal. Coronal sections of the brain revealed definite petechial hemorrhages scattered throughout the posterior portion of the corpus callosum on each side. These hemorrhages were almost 1 mm. in diameter. A few similar petechiae were found scattered throughout the white substance of the brain, particularly in the left occipital lobe. The middle ears and mastoids were normal.

Microscopic Examination: Sections of the brain reveal numerous small areas of hemorrhage, all of which show marked hemolysis. No

definite red cell outlines are visible in any of these hemorrhagic areas. The relation of these petechiae to the small vessels cannot be determined because of the hemolysis in the areas. The cerebral cortex and the meninges are normal.

Comment: Cerebral hemorrhages following scarlet fever have been reported by various investigators. Southard and Sims in 1904 studied a case both clinically and pathologically. Their patient, who was 5 years old, developed cerebral symptoms 7 weeks after the onset of the acute exanthem. At autopsy a large number of hemorrhagic areas which extended down through the cortex into the white substance of both parietal lobes were found. Microscopically numerous large petechiae were visible in the gray substance of the parietal lobes. Surrounding these hemorrhages there were numerous phagocytes which were invading the hemorrhagic areas and taking up the polymorphonuclears, red blood cells and fat. The nerve cells showed various degenerative changes. Signs of frank suppuration were also found in these necrotic areas. Toomey, Dembo and McConnell in 1923 described a similar case. In their case, however, the brain appeared normal externally, although both ventricles were filled with blood. On section there were numerous hemorrhages into the basal nuclei. Microscopically many of the blood vessels were thrombosed and there was a destruction of the vessel wall resulting in the perivascular extravasations.

Purpura Hemorrhagica

A primary or secondary purpura may produce cerebral hemorrhages of sufficient severity to cause death. These purpuras almost always show, aside from the cerebral lesions, signs of bleeding elsewhere in the body. Such widely distributed hemorrhages, together with the other findings of purpura, should enable one to arrive at the correct diagnosis of the underlying condition. Intracranial hemorrhages in purpura have been reported by Duplay, Jousset, Duplaix, and Alpers and Duane. All of their cases presented other characteristic clinical features of purpura, aside from the terminal cerebral bleeding. Recently we have had occasion to study one such case.

CASE 1. The patient, aged 36 years, had been treated for renal calculi, pyelonephritis and cystitis. He had had multiple stones removed from the left ureter and from the bladder. A few days before admission to the hospital he was suddenly seized with pain over the left kidney. This pain radiated down the left

side to the scrotum. It was accompanied by chills, fever and a little urgency and burning on urination. On admission to the hospital examination revealed a blood pressure of 90/60 and a temperature of 99° F. A cystoscopic examination showed a slight cystitis with no return of indigocarmine from either ureter in 15 minutes. The urine contained much pus, erythrocytes and epithelial cells. Roentgen-ray studies revealed a right pyelonephritis and a left ureteral calculus.

Shortly after admission the patient developed severe epistaxis, for which he was given hemostatic serum. Nevertheless, he not only continued to bleed from the nose but began to bleed also from the mouth, bladder, rectum and finally into the subcutaneous tissues. A spinal tap at this time revealed a blood-tinged fluid. The patient's respirations gradually became irregular. Shortly before his death the hemorrhage from the mucous surfaces became very profuse.

Laboratory studies showed a red blood count of 3,560,000, hemoglobin 70 per cent, non-protein nitrogen 163 mg. per 100 cc. of blood, creatinin 3.2 mg. per 100 cc. of blood, coagulation time of 7 minutes, bleeding time 12 minutes, and platelet count 184,000. His temperature varied from 99° to 100.2° F.

Autopsy: The body was covered with numerous petechiae situated beneath the skin and varying in diameter from 1 mm. to 1 cm. There were small ecchymotic areas in the sclerae of both eyes. Petechial and ecchymotic areas were also observed over the serous surface of the heart and in the kidneys, liver and bladder. Aside from these hemorrhages there was a bilateral pyelonephritis, with renal calculi filling the entire pelvis of the left kidney.

The surface of the brain was covered with a thin film of blood. There was a clot measuring 2.5 cm. in diameter in the anterior fossa under the right frontal lobe. A softened area was observed in the right frontal lobe anterior to the lenticular nucleus. Scattered through this region were numerous old and new hemorrhages. There was an extensive hemorrhage 6 by 4 by 4 cm. in the left occipital lobe. This lesion extended into the lateral ventricle medially, and to the external surface of the brain laterally. There was extensive destruction of brain tissue throughout this massive hemorrhage. In the dentate nucleus of the right cerebellum there was a hemorrhage measuring 2.5 by 2.3 by 2 cm.

Microscopic Examination: This reveals extensive hemorrhages and destruction of the cerebral tissue (Fig. 14). Massive diffuse extravasations of red cells have infiltrated the cerebral tissue, causing much destruction both in the white and gray substance (Fig. 14). These hemorrhages do not involve the entire brain but are localized in the various regions listed, causing much local damage. They appear to be fresh and there is no reaction on the part of the supporting elements of the brain. No fat granule cells or rod cells can be found

in the damaged areas. Throughout the tissue not involved in this massive bleeding there are occasional small, discrete, perivascular hemorrhages. These vary in size from just a few erythrocytes to a fairly large extravasation that not only fills the perivascular space but also extends into the surrounding tissue. The red cells in these areas of hemorrhage are very peculiar in appearance, most of them being fragmented and elongated. No alterations of any kind can be detected in the vessel walls. No thrombi are observed in the vessels. The nerve cells appear uninjured.

Traumatic Encephalitis

The marked increase in automobile accidents has elevated the problem of trauma to the head to one of major importance. Among these traumatic lesions there are many that are diagnosed cerebral concussion or contusion in which no fracture of the skull has occurred, and from which the patient may die or from which he may recover with residual complaints of an indefinite nature. Many investigators have studied these brains and have observed definite organic lesions in many of them (Bennett and Hunt, Osnato and Giliberti, Cassasa, Jefferson, Trotter, Head). Osnato and Giliberti in a careful examination of a series of cases which terminated fatally found numerous diffuse hemorrhages scattered throughout the brain tissue. There was no reaction on the part of the cellular elements. They suggested the term "traumatic encephalitis" for these lesions. Similar changes have been reported by Cassasa.

We have had the opportunity to study such a case of cerebral trauma following an accident, in which no skull fracture had occurred and in which the gross sections of the brain showed a resemblance to the true hemorrhagic encephalitis (Fig. 16).

CASE 1. A male, aged 45 years, was injured in an automobile accident and died after 4 days of unconsciousness. At autopsy numerous abrasions were observed over the face and scalp. There were fractures of numerous ribs on both sides but no skull fracture. A slight amount of terminal pneumonia was present bilaterally, although most of the lung tissue was fairly normal. The other internal organs appeared normal.

On removal of the brain a considerable amount of subdural hemorrhage was found in the right posterior fossa. Careful sectioning of the brain revealed numerous discrete hemorrhages scattered through the entire brain substance but more prominent in the white

matter (Fig. 16). Close scrutiny of the sections showed small traumatized areas distributed through the brain. These contained softened hemorrhagic brain tissue and were constantly situated near the surface. These lesions were suggestive of a traumatic origin.

Microscopically, numerous perivascular hemorrhages of both the ring and the solid type are seen throughout the brain tissue. These hemorrhages are most numerous in the white substance, although many are also observed in the cortex. The cortical hemorrhages are often of a diffuse nature, extending to the surface of the brain. There is no tissue injury about the perivascular hemorrhages and no reaction on the part of the cerebral elements. In the traumatized regions there is extensive tissue destruction together with some hemorrhage, the former constituting the most prominent part of the picture. The injury involves not only the entire cortical substance but often extends into the subcortical regions.

It seems from the above protocol that in some cases of cerebral concussion or contusion, the pathological findings resemble somewhat those of hemorrhagic encephalitis, but the history of a pre-existing head injury, the external signs of severe bruising of the body, and a careful examination of the brain for traumatized areas usually permits a correct diagnosis.

Fat Embolism

Fat embolism of the brain may follow fracture of various long bones. It produces cerebral petechiae which grossly might be confused with hemorrhagic encephalitis. A fat stain, however, readily demonstrates the tiny fat emboli filling the vessels about which the hemorrhage has occurred.

Hemorrhagic or Venous Infarct

Although hemorrhagic or venous infarcts are comparatively rare, they should be mentioned briefly in this discussion, since upon cursory examination they might be confused with those cases of true hemorrhagic encephalitis in which extensive extravasations have occurred. Venous infarction was described as early as 1913 by Oeller, who called the condition "hemorrhagic encephalitis." He considered it an inflammatory thrombosis of the smaller or larger veins of the brain with resulting rupture of the capillaries because of increased

arterial pressure. Recently we have had occasion to study such a case in a 27 year old female who was admitted to the hospital because of a pelvic inflammatory condition. After a short stay, she suddenly developed hemiplegia of the left side of the body. She gradually lapsed into coma and died. At autopsy a small circumscribed abscess containing dark brown necrotic material was found near the hilum of the right lung. A small fresh infarct was present in the upper pole of the spleen. The left tube and ovary were fused to form a large mass, which on section contained a small amount of dark, thin, purulent material. Gross examination of the scalp and calvarium was negative. On removing the dura a large thrombosed vein was seen extending along the left hemisphere from the Sylvian fissure, posteriorly and downward to the occipital pole of the brain. The brain tissue immediately beneath this vessel was hemorrhagic and necrotic. Coronal sections of the brain revealed numerous softened hemorrhagic areas involving the entire cortex and subcortical tissue along the course of this large thrombosed vein. There were no hemorrhages found elsewhere. Microscopic examination shows numerous thrombosed pial veins with many petechial hemorrhages in their immediate neighborhood; inflammatory changes are already evident within many of these degenerating regions.

The presence of thrombosed veins and the absence of hemorrhages and necrosis, except about these injured veins, is sufficient to distinguish this condition from true hemorrhagic encephalitis.

ETIOLOGY OF ENCEPHALITIS

The etiology of many types of encephalitis is still undetermined in spite of the extensive experimental work that has been done. The scope of the present paper does not warrant a complete discussion of the very numerous and widely scattered reports on the etiology of encephalitis. It can be stated at the outset, however, that with few exceptions no conclusive evidence has been presented to show that encephalitis in man is due to a virus infection, a cultivatable bacterium, or a toxicosis, although these are the three prevailing views. Most investigators, at the present time, tend to favor a virus infection as the etiological factor in many types of human encephalitis, although there has developed a marked difference of opinion as to how this agent really acts. The main trends of thought are in two

directions. One group of investigators believes that the cerebral involvement in encephalitis is due to a specific, neurotropic, invisible, but as yet undiscovered virus infection (Stern, von Economo, McKinley, and Flexner in epidemic encephalitis; Bijl and Frenkel, Gildemeister, Turnbull and McIntosh, Flexner, and Scott in post-vaccination encephalitis; Pette and Wohlwill in measles encephalitis; Gordon in mumps encephalitis). The other school maintains that some environmental factor or infectious disease breaks down the natural resistance of the individual and thus predisposes him to an infection by some non-specific and often detectable virus. Levaditi, Doerr and Schnabel, Berger, Doerr and Zdansky, Gay and Holden advocate a herpes virus in epidemic encephalitis; Loewe and Strauss, immobile "globuli" in epidemic encephalitis; Gay and Holden, a herpes virus in measles encephalitis; Strümpell, the poliomyelitis virus in epidemic encephalitis.

During the course of this study fresh brain tissue was obtained from a case of hemorrhagic encephalitis (Case 20). In coöperation with Dr. C. Buggs from the department of bacteriology, a virus was isolated from this tissue and was carried through twelve serial transmissions in rabbits by means of intracerebral inoculation. The infectious brain tissue does not affect mice or guinea pigs.

The micropathological alterations observed in the rabbit brains resemble in many details those changes found in the human cases of hemorrhagic encephalitis. So far a detailed examination for inclusions has not been instituted.

A detailed description of the experimental studies in rabbits will be given in a separate publication.

Twenty cases of hemorrhagic encephalitis have been studied at postmortem. A brief report of each of these follows.

CASE REPORTS

CASE 1. Male, aged 33 years, apparently well until 10 A.M. on the day of his death when he was found unconscious at work. He was taken to the hospital where he had repeated attacks of generalized convulsions. He died 5 hours after the onset of the illness without regaining consciousness.

Autopsy: Negative with the exception of the brain. The left lateral ventricle contained a slight amount of blood. Numerous petechial hemorrhages could be seen in the region of this ventricle and in the pons. A few larger diffuse hemorrhages were also present.

Microscopic Examination: Petechiae are found only in those areas in which hemorrhages are visible on gross examination. These hemorrhages are mainly perivascular. Around some of the larger hemorrhages there is a slight amount of tissue destruction but no fat granule cells are seen. An occasional small vessel in the pons is surrounded by a few mononuclear cells.

CASE 2. Female, aged 25 years, found unconscious and taken to the hospital. She showed no evidence of paralysis, although she was quite spastic and had attacks of epileptiform convulsions. Death occurred shortly after admission to the hospital.

Autopsy: Negative with the exception of the brain. Small hemorrhages were visible throughout the brain tissue. Numerous small hemorrhages were scattered through the white substance of the brain and a few were localized in the basal nuclei.

Microscopic Examination: These hemorrhages are both perivascular and disseminated. No cellular infiltration is visible.

CASE 3. Male, aged 41 years, was found unconscious at work and died about 4 hours later. He had been working regularly up to the onset of his attack. The spinal fluid was bloody.

Autopsy: Negative except for the brain. The entire under-surface of the brain was covered by a fine film of blood. Careful examination of the surface of the brain revealed no contusions or ruptured vessels. On sections of the brain numerous petechiae were found in the pons and medulla and around the ventricles.

Microscopic Examination: The hemorrhages are small and localized around the blood vessels. In sections through the pons particularly, almost every small vessel is surrounded by a wide zone of red cells. No cellular infiltration is observed within the brain tissue.

CASE 4. Female, aged 36 years, was found one morning dead in bed. She had been in apparent good health.

Autopsy: Negative except for the brain, sections of which revealed many small hemorrhages scattered throughout the white matter of the cerebral hemispheres.

Microscopic Examination: The hemorrhages are mostly perivascular. No cellular infiltration is visible.

CASE 5. Male, aged 40 years, died suddenly while sitting in a barber's chair.

Autopsy: Negative except for the brain. (Careful examination of the coronaries revealed no lesions.) Grossly the scalp, skull, meninges and brain showed no conspicuous lesions.

Microscopic Examination: Multiple small hemorrhages are scattered diffusely through the cerebral hemispheres. Occasionally the hemorrhages are arranged perivascularly. No marked cellular infiltrations are visible.

CASE 6. Female, aged 14 months, had always been in good health. She was playing in the house when she was suddenly seized with generalized convulsions occurring at approximately 3 minute intervals. She was taken to the hospital where she died 3 hours after the onset of the illness.

Autopsy: Negative, except for the brain which was congested and on section revealed numerous pin-point hemorrhages scattered chiefly throughout the occipital lobes, the temporal lobes and the basal nuclei.

Microscopic Examination: These hemorrhages are both diffuse and perivascular in distribution. No cellular infiltration is apparent. The spinal cord is negative both grossly and microscopically.

CASE 7. Male, aged 25 years, developed a cold about 1 month before his present illness from which he apparently had recovered. He was well until 5 days before his death when he suddenly developed nausea, vomiting and dizziness. These symptoms persisted, and 2 days later he became irrational and stuporous. This stupor deepened into a coma which lasted until his death. The blood and spinal fluid Wassermann were negative.

Autopsy: Aside from the presence of a horseshoe kidney, the findings were negative with the exception of the brain. The external surface of the brain was normal, but on section numerous small pin-point hemorrhages were detected throughout the white matter of the cerebral hemispheres.

Microscopic Examination: The small hemorrhages are mostly perivascular, consisting of rather dense collars of blood cells surrounding the smaller vessels and extending into the surrounding brain tissue. The vessels are also filled with erythrocytes. No cellular infiltration is visible. Cultures of the brain and of the heart muscle are negative.

CASE 8. Male, aged 44 years. Illness started 2 weeks before death with a severe headache and general malaise. The headaches increased in severity and he gradually became listless, irrational, and finally stuporous. He perspired profusely. A physical examination was performed shortly before the onset of stupor.

His face was flushed and the facial expression was dull. He responded slowly when spoken to, giving fairly intelligent replies; however, frequently during the examination, he dropped into a semicomatose condition from which he could easily be aroused. The pupils were irregular, the right one did not react to light, while the left one reacted but slightly. There was a coarse tremor of the tongue and the neck was slightly rigid. His reflexes varied, being normal on one day and hyperactive on the next. The Babinski sign also varied from day to day. The red blood count was 5,136,000; white blood count 9500; polymorphonuclears 83 per cent, lymphocytes 17 per cent. The blood and spinal fluid Wassermann reactions were negative. The spinal fluid was under increased pressure, and showed 10 cells per cmm.

His condition gradually grew worse. The temperature rose from 101 to 106° F., the coma deepened and he died 2 weeks after the onset of the illness.

Autopsy: Negative except for the brain. Grossly there was a reddish tinge to the brain tissue in the regions of the frontal lobe, the temporal lobe and the basal nuclei. No hemorrhages were visible.

Microscopic Examination: All the vessels of the brain are markedly distended with blood. Surrounding the vessels, chiefly in the frontal and temporal lobes, are numerous perivascular hemorrhages. Occasionally areas of hemorrhage apart from vessels are seen. These are most numerous in the frontal and temporal lobes. No cellular infiltration is visible.

CASE 9. Male, aged 18 years, always well and active. He suddenly began to complain of occipital headaches and dizziness. Shortly afterwards he developed a marked weakness of the left forearm and hand and of the right leg. There was twitching of the involved muscles. The patient became very restless. His reflexes remained normal. The temperature gradually rose, and he died 2 days after the onset of illness.

Autopsy: Negative except for the brain which contained numerous small hemorrhagic areas.

Microscopic Examination: Many small perivascular and diffuse hemorrhages are found throughout the white matter. Small areas of degeneration are visible in the white matter, especially about the periphery of the hemorrhages, but only slight cellular reaction has as yet occurred. Occasional vessels are surrounded by mononuclear cells.

CASE 10. Male, aged 35 years. The first indication of illness was a marked personality change followed shortly by weakness and drowsiness. He became irrational, his muscles became spastic, and he developed generalized convulsions. The red blood cell count was 4,150,000, hemoglobin 80 per cent, the white blood count 8000. The urine was negative. A spinal tap showed a clear fluid under in-

creased pressure. There were 35 cells per cmm. The Wassermann tests were negative. The temperature rose rapidly and he died 4 days after the onset of illness.

Autopsy: Negative except for the brain. On section the brain showed many small hemorrhages in the white matter.

Microscopic Examination: The hemorrhages are both perivascular and diffuse. Some of the larger ones are surrounded by damaged brain tissue. Numerous scavenger cells have already invaded these demyelinated regions. A few of the vessels are surrounded by cuffs of mononuclears.

CASE 11. Male, aged 14 years. Illness started suddenly with headache and vomiting. A few hours after the onset of this illness there occurred generalized convulsions and the patient lapsed into unconsciousness. His temperature at this time was normal. On the following day it had risen to 101° F. and the convulsions persisted. There was never any neck rigidity, the Kernig sign remained negative. A spinal tap showed a slightly bloody fluid. The temperature gradually rose to 106.5° F. and the patient died 3 days after the onset of illness.

Autopsy: Negative except for the brain. The vessels over the surface of the brain were distended. There was a small amount of free blood in the subarachnoid space around the base of the brain. Sections of the brain revealed numerous pin-point hemorrhages scattered throughout the white and gray substance. Many of the bleeding points were near the surface.

Microscopic Examination: The small hemorrhages are mostly perivascular. There is an occasional petechia in the cortex. The basal nuclei are moderately involved. Some of the vessels are surrounded by a few mononuclear cells. Many of the large nerve cells of the cerebral cortex and of the basal ganglia show chromatolysis and pyknosis of their nuclei.

CASE 12. Female, aged 36 years. Illness first started with the complaint of headache. When her husband returned from work that evening, he found her unconscious but not paralyzed. She was immediately removed to the hospital.

The physical examination revealed very few findings. The pupils were equal. The right upper extremity was spastic, while the left upper and right lower extremities were flaccid. The blood pressure was 110/80. The urine examination was negative. The spinal and blood Wassermann tests were negative. During her stay in the hospital she never regained consciousness. Her pulse varied between 130 and 150 and her temperature between 102°-108° F. She died 3½ days after the onset of illness.

Autopsy: Negative except for the brain, which showed many petechial hemorrhages throughout the white matter. These hemorrhages were also very numerous in the basal nuclei.

Microscopic Examination: The hemorrhages are both free and perivascular in type. No cellular infiltration or nerve cell degeneration is visible.

CASE 13. Male, aged 3 years. Had always been very robust, and before the onset of his present illness showed no signs of any infection. While eating bread one day, he complained of feeling ill. His eyes became fixed, his arms and legs showed a marked spasticity and his head became stiff and retracted. Drowsiness gradually set in and he died about an hour after the onset of illness. No one else in the family eating the same bread became ill.

Autopsy: Negative except for the brain. The vessels in the brain were prominent. On section it was difficult to determine whether we were dealing with dilated vessels or true hemorrhages. Most of the lesions seemed to be in the temporal and occipital lobes of the brain, involving the gray matter as well as the white.

Microscopic Examination: Most of the small vessels of the brain are distended with blood. True hemorrhages of the perivascular type are found only in the temporal and occipital regions involving both the gray and white matter. A few small mononuclear cells are found around some of the dilated vessels.

CASE 14. Male, aged 5 years, had apparently always been in good health. He was suddenly taken ill with headache, and malaise. A physician was called, who found the boy moderately ill. His temperature was 101° F., pulse 120, and there were no signs of an upper respiratory infection. A few hours after the onset of illness, the child's neck became stiff and he experienced slight difficulty in swallowing. His temperature gradually rose to 104° and he died 9 hours after the onset of the illness.

Autopsy: Negative except for the head. Numerous small hemorrhages were scattered throughout the base of the brain, the pons, and the upper part of the medulla.

Microscopic Examination: The hemorrhages are both diffuse and perivascular. No polymorphonuclear cells are discovered. There is no meningitis.

CASE 15. Female, aged 55 years. She had been well until the onset of the present illness. While working in her garden she experienced a severe headache. A physician was called who found her temperature to be 102° F. The next day she was restless and had a severe convulsion which consisted of spasmodic contractions of the muscles of all her limbs. She soon lapsed into unconsciousness and

was admitted to the hospital in this condition. At that time her pupils reacted sluggishly to light, but the ophthalmoscopic examination revealed normal findings. The blood pressure was 108/64. The urine was normal. A spinal puncture showed the fluid under increased pressure, with 50 cells per cmm. Her temperature gradually rose to 105° F., the coma deepened, she had occasional tonic convulsions and died 6 days after the onset of illness.

Autopsy: The gross appearance of this brain is described in detail elsewhere in this paper.

Microscopic Examination: Most of the hemorrhages are perivascular, although some larger diffuse ones can be observed extending up into the cortex. There is marked tissue destruction around the larger hemorrhages. There is perivascular demyelination around vessels not surrounded by erythrocytes. Reactions on the part of the microglia and neuroglia are readily visible. There is no lymphocytic infiltration into the tissue.

CASE 16. Female, aged 11 years, had always been well until the present illness which started with a severe headache. She became feverish and complained of extreme aching in her bones, back and head. These complaints continued for 2 days, when she suddenly became unconscious and had what her mother described as a series of generalized convulsions which lasted for 1 hour. From that time on she did not regain consciousness. She had athetoid movements of the arms and hands. Her legs were alternately spastic and flaccid. On admission to the hospital the child was in a deep coma. The lower extremities were spastic while the upper were athetotic. The knee jerks were exaggerated; there was an unsustained ankle clonus; and the Babinskis were bilaterally positive. A spinal puncture showed no increased pressure; there was a pleocytosis. The urine was negative. The red blood count was 4,600,000, hemoglobin 83 per cent, white blood count 14,000, with 91 per cent polymorphonuclears and 9 per cent lymphocytes. The Wassermann test was negative.

The child's signs and symptoms varied greatly during hospitalization. The reflexes at one time would be exaggerated and the extremities spastic, while a few hours later the reflexes would be decreased or absent and the extremities flaccid. Her temperature rose to 107° F., the pulse increased to 140, the coma continued, and the child expired 7 days after the onset of illness.

Autopsy: The lungs showed a slight edema and a beginning pneumonia. Otherwise the findings were negative except for the brain, which on section revealed numerous hemorrhages throughout the white substance, being most marked in the occipital regions.

Microscopic Examination: The hemorrhages are both diffuse and perivascular in arrangement. Numerous vessels show marked perivascular demyelination with the characteristic reactions on the part of the scavenger and glial cells. No involvement of the cortex is observed.

CASE 17. Female, aged $2\frac{1}{2}$ years. She had always been well. Her illness first manifested itself with a slight restlessness and a moderate elevation of temperature. A few hours later she developed generalized convulsions which continued until the time of death, 12 hours after the onset of illness.

Autopsy: Negative except for the brain. There was an engorgement of the pial vessels. Sections of the brain suggested small hemorrhages throughout the white matter.

Microscopic Examination: There is a marked distention of the cerebral vessels, many of which are surrounded by a collar of erythrocytes which in some cases extend beyond the perivascular spaces and into the brain substance. A few small vessels are surrounded by mononuclear cells. Only an occasional polymorphonuclear is detected in and around the vessels. There is no change in the ganglion cells. The cord is negative.

CASE 18. Male, aged 20 years. During the past few days his family noticed that he had been lying down quite frequently and sleeping more than usual. However, since he had always been healthy no attention was paid to this lack of spirit and exaggerated fatigue. He continued to work hard, until one morning he suddenly developed generalized convulsions. When the doctor arrived the patient was conscious and complained of severe headache. He seemed somewhat dull mentally. His temperature and pulse were normal. Three hours later he again had a generalized convulsion which lasted only a few minutes. At this time he was in a semistuporous state, and his temperature was somewhat elevated. He soon lapsed into unconsciousness from which he did not recover. There was no neck rigidity and the extremities were slightly spastic. The leukocyte count was 11,000, urine was normal, and the blood pressure was normal. A spinal tap revealed a bloody fluid under increased pressure. A detailed examination of the fluid and animal inoculation revealed no organisms. By evening the patient's temperature had risen to 104.2° F., pulse 150, and respirations 20. The temperature then varied from 102° – 104° F. until the time of death, which occurred the following morning about 1 day after the acute onset, or 3 days after the onset of fatigue and drowsiness. The spinal fluid obtained a few hours before death was identical with the first specimen.

Autopsy: Negative except for the brain. The surface of the brain in the right frontal and parietal regions had a reddish appearance. There was a reddish tinge to the spinal fluid at the base of the brain. Sections of the brain revealed a slight amount of pinkish fluid in the right lateral ventricle. Throughout the white substance there were numerous hemorrhages varying in size from those barely visible to some a few mm. in diameter. Some of the largest hemorrhagic areas were in the right frontal and parietal regions where the involvement

extended through the cortex to the surface of the brain. There was no involvement of the cerebellum.

Microscopic Examination: The hemorrhages are both diffuse and perivascular. In many areas, especially in the right frontal and parietal regions, large hemorrhages are seen with many smaller ones surrounding them. The brain tissue in and around the larger hemorrhages is destroyed. A fat stain reveals macrophages invading the necrotic tissue. The hemorrhages are numerous in the region of the right lateral ventricle. Many of the vessel walls are damaged and erythrocytes can be seen which have broken out of the vessels into the perivascular spaces. A few polymorphonuclears are discovered scattered among the larger areas of hemorrhage and destroyed brain tissue. No changes can be discovered in the large nerve cells except in the vicinity of the hemorrhages. Occasional areas showing perivascular demyelination are observed.

Smears and cultures of the brain remained sterile.

CASE 19. Male, aged 10 years. He had always been well. The first signs of illness were chills and vomiting. He was put to bed and for the next few days seemed to improve. On the third day of his illness he complained of headache. That evening he was very irritable and drowsy and by morning could not be aroused. He was admitted to the hospital in this comatose state. Physical examination soon after admission revealed a poorly nourished, unconscious boy. There was no neck rigidity, ocular reflexes were normal and the fundi were normal. The lungs, heart and abdomen were negative. A definite rigidity was noted in moving the upper extremities. None of the deep reflexes was obtained. His temperature was 106.8° F. The white blood count was 21,100 with 81 per cent polymorphonuclears. The urine examination was negative. The spinal fluid was essentially normal except for 10 cells per cmm.

During his stay in the hospital it was observed that the neurological findings were extremely variable. At times the limbs were flaccid, while a few hours later they would be spastic. The reflexes varied in the same manner. His temperature varied, usually remaining above 106°. His respirations became more labored and he died 2 days after admission to the hospital and 4 days after the appearance of the first symptoms.

Autopsy: Entirely negative except for the brain. There was no evidence of blood in the cerebrospinal fluid. At the base of the brain over the pons, medulla and adjacent interstices there was a slight hemorrhagic film. The surface of the brain showed no further hemorrhages. On section numerous petechiae were visible throughout the basal ganglia, the cortical regions and the central white matter. The latter showed a slight predominance of lesions. These hemorrhagic areas could have been easily confused with dilated vessels.

Microscopic Examination: This shows that many of the suspicious areas are true hemorrhages, while others are merely dilated vessels. Most of the actual petechiae are situated in the white substance of the frontal and temporal regions. Numerous hemorrhages are detected in the pons and medulla also. Only in these latter areas is any round cell infiltration visible. No polymorphonuclears can be found. An occasional ganglion cell seems to be undergoing degeneration, but this finding is too infrequent to be of significance.

CASE 20. Female, aged 7 months. She was first taken ill with a cold. That same evening she lapsed into coma, and her temperature started to rise. She was admitted to the hospital where examination revealed a comatose infant with a temperature of 104.2° F., a slight neck rigidity and normal reflexes. The rest of the physical examination was negative. There were no signs of an upper respiratory infection. The urine was normal and a spinal tap showed the fluid to be under increased pressure with 560 cells per cmm. No bacteria could be found in the smears. Repeated spinal taps showed great numbers of white cells. The red blood count was 2,160,000; white blood cells were 25,000 with 71 per cent polymorphonuclears.

The coma persisted. At times she would have clonic contractions of the extremities most marked on the right side. The reflexes varied and her temperature remained high. She died about 2 weeks after the onset of the illness.

Autopsy: The gross appearance of this brain is described in a previous paragraph.

Microscopic Examination: The greatest damage is in the left half of the cerebrum, although numerous changes are also seen on the right side. The most marked involvement is in the white substance which contains numerous hemorrhages. These are visible in all sections of the brain but are most extensive in the left frontal and parietal lobes where large areas of brain tissue are completely replaced by red blood cells. Some of the blood vessels are distended with erythrocytes and many more contain collars of red cells. An extensive proliferation of the endothelium of many of the small blood vessels is noted in the frontal regions and around the left lateral ventricle. Destruction of the brain substance is noticeable about many of the larger hemorrhages, as well as around the blood vessels. In these areas there is a great proliferation of the microglia. Sections of the basal nuclei are normal, with the exception of a few dilated vessels. An occasional small hemorrhage is detected in the internal capsule. The pons, medulla, cerebellum and cord are all normal.

Smears and cultures of the brain revealed no growth. Animal experimentation with this brain tissue has been described above.

TABLE IV
Summary of Cases

Case No.	Sex	Age	Duration of illness	First symptoms
1.....	M	yrs. 33	5 hours	Unconsciousness Convulsions
2.....	F	25	12 hours	Unconsciousness Convulsions
3.....	M	41	4 hours	Unconsciousness
4.....	F	36	few hours	Found dead
5.....	M	40	few minutes	Sudden death
6.....	F	14 mo.	3 hours	Convulsions
7.....	M	25	5 days	Dizziness Stupor
8.....	M	44	14 days	Headache Stupor
9.....	M	18	2 days	Headache Restlessness
10.....	M	35	4 days	Malaise Stupor
11.....	M	14	3 days	Headache Convulsions Unconsciousness
12.....	F	36	3½ days	Headache Unconsciousness
13.....	M	3	1 hour	Malaise Stupor
14.....	M	5	9 hours	Headache Malaise
15.....	F	55	6 days	Headache Convulsions Unconsciousness
16.....	F	11	7 days	Headache Convulsions Unconsciousness
17.....	F	2½	12 hours	Restlessness Convulsions
18.....	M	20	3 days	Headache Convulsions Stupor
19.....	M	10	4 days	Headache Stupor
20.....	F	7 mo.	14 days	Stupor

SUMMARY AND CONCLUSIONS

1. Twenty cases of a peculiar involvement of the central nervous system are reported. We have called this disease hemorrhagic encephalitis, since clinically the patients present a typical picture of encephalitis and pathologically the most striking lesion is a hemorrhagic involvement of the brain.

2. Hemorrhagic encephalitis may be characterized clinically as an acute ailment of the central nervous system occurring in previously healthy young individuals, and manifesting itself by a sudden onset, headache, an abrupt rise of temperature and a rapid loss of consciousness. Convulsions are common, the extremities are spastic, and the reflexes are frequently abnormal and variable. Death ensues in from a few hours to several days after the onset of the illness.

3. The most conspicuous feature of this disease is the pathological picture in the brain. It is chiefly hemorrhagic and predominantly in the white matter. The hemorrhages vary widely in number and size, from extensive extravasations which destroy much brain tissue to tiny perivascular bleedings. In the brains of patients who survive the first few days there are often observed areas of non-hemorrhagic perivascular demyelination which are invaded by scavenger cells. Consistent specific changes in the ganglion cells have not been observed. An occasional blood vessel shows a slight perivascular infiltration of mononuclears. A few widely scattered polymorphonuclears can be detected in the areas of degeneration. Postmortem study shows that all the organs except the brain are normal.

4. Cerebral hemorrhages may frequently be found in other diseases but by careful clinical and pathological study these can easily be differentiated from true hemorrhagic encephalitis.

5. The brain tissue from 1 case of hemorrhagic encephalitis has proved virulent to rabbits on intracerebral inoculation.

6. On the basis of the present study we are inclined to accept hemorrhagic encephalitis as a clinical entity and believe it is entitled to a definite position in neurological nosology.

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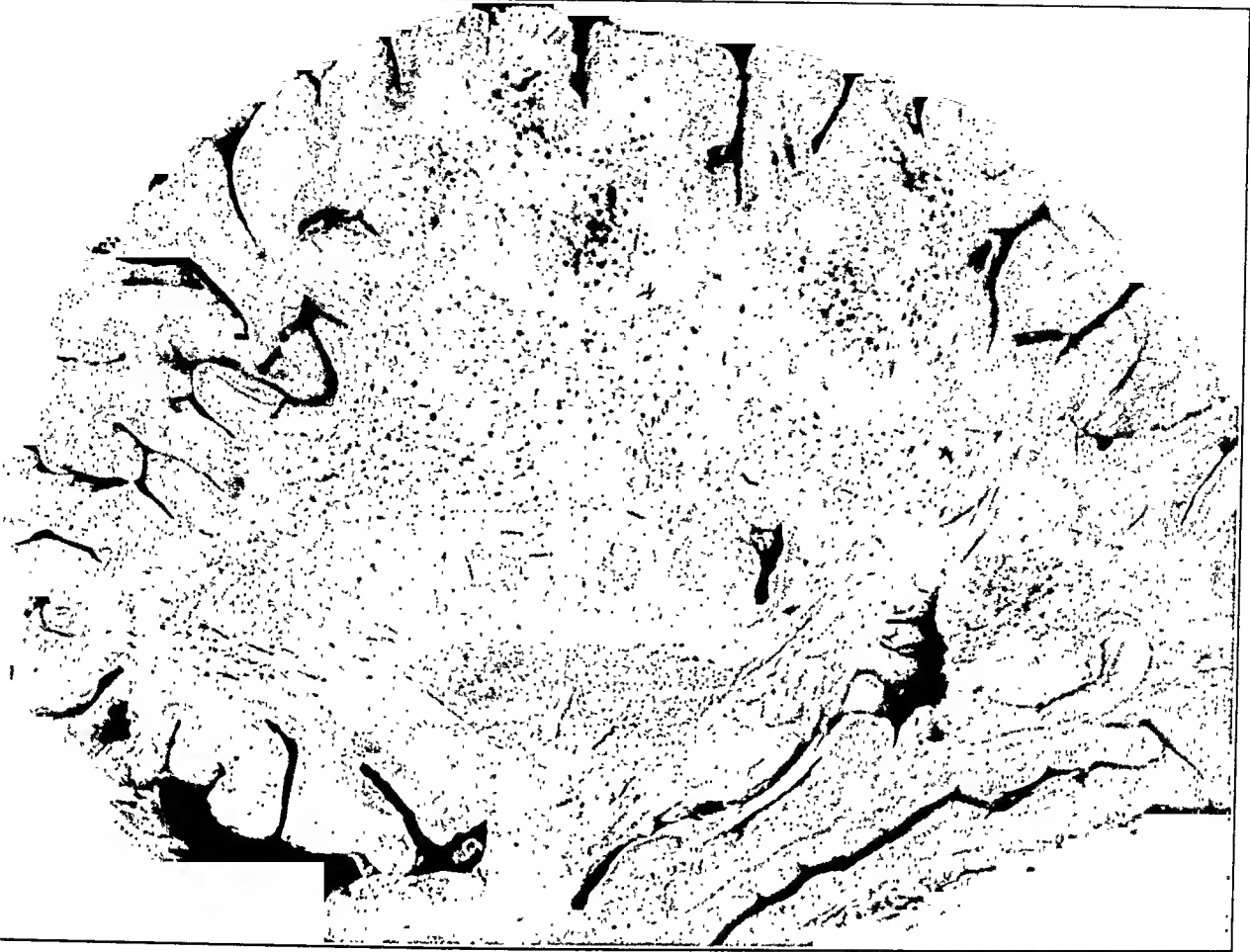
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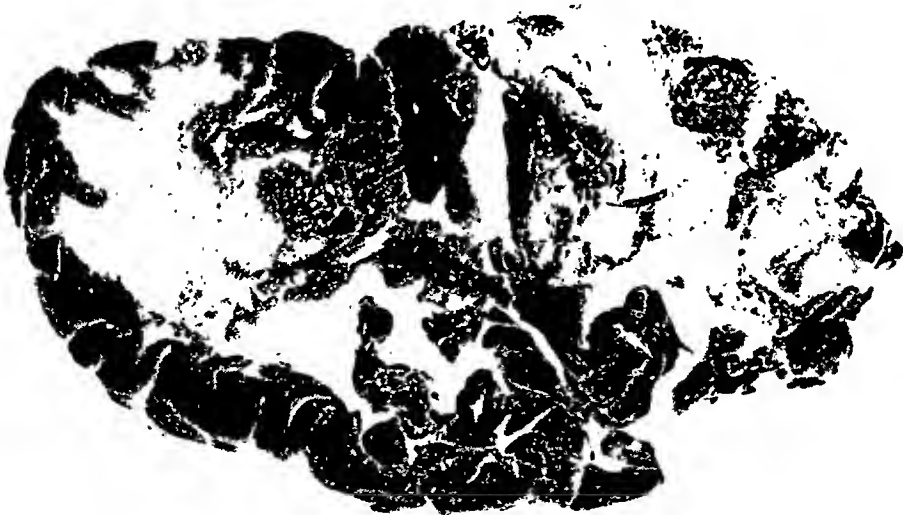
DESCRIPTION OF PLATES

PLATE 26

- FIG. 1. Section through the brain of a 55 year old female dead of hemorrhagic encephalitis (Case 15). The hemorrhages are almost exclusively of a small discrete type. Note their sharp localization to the white substance of the brain and their relative paucity in the basal nuclei. $\times 0.8$.
- FIG. 2. A case of hemorrhagic encephalitis in a child aged 7 months (Case 20). Sections show massive irregular hemorrhages and a marked destruction of brain tissue. The necrotic brain tissue has extended through the cortex to the surface of the brain. $\times 0.8$.



I



METRIC 2 3 4 5 6 7 8 9 10

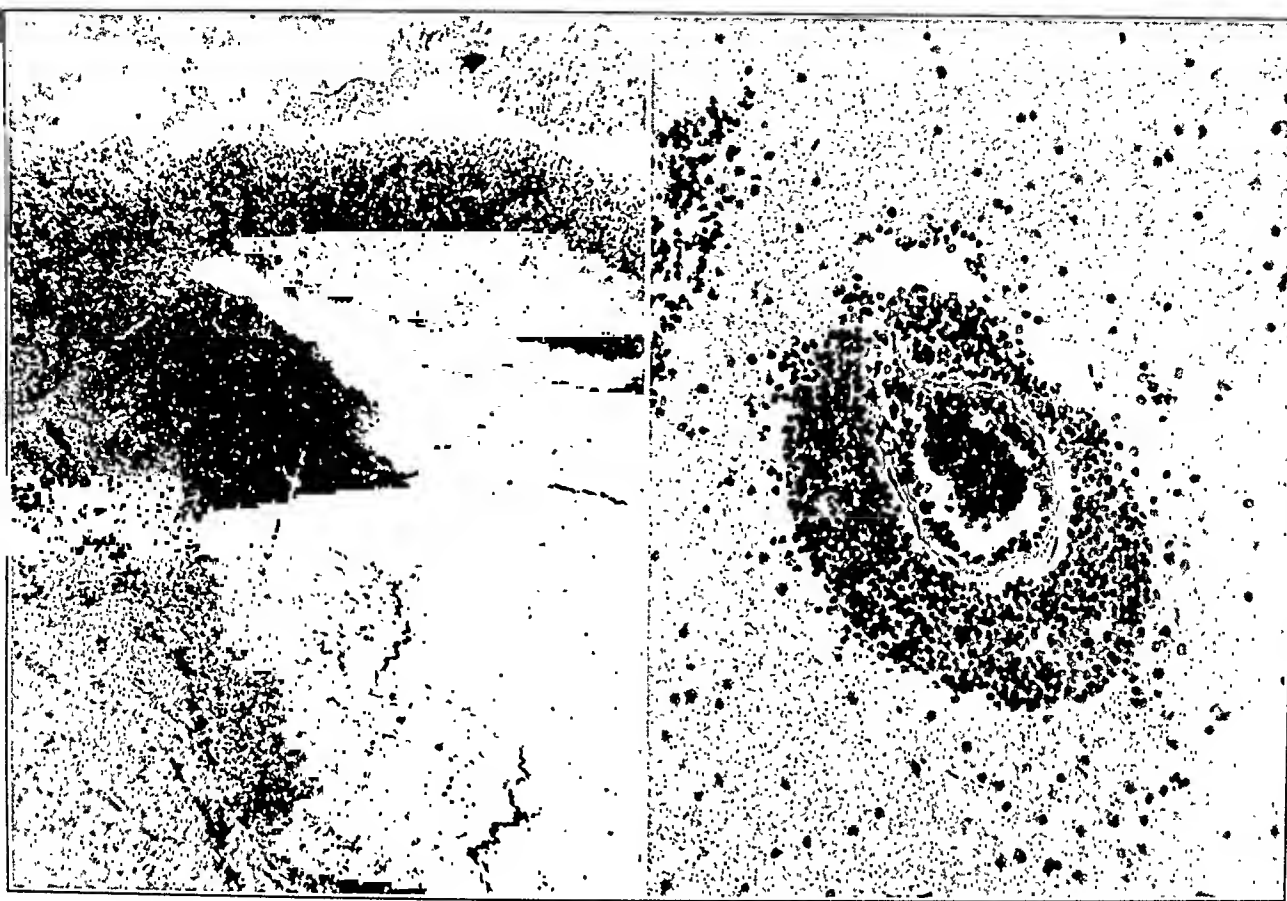
PLATE 27

FIG. 3. A large hemorrhage of the brain from a case of hemorrhagic encephalitis. The red blood cells have extended to the surface of the brain. Numerous smaller extravasations of red cells can be detected around the larger one. Azocarmine stain. $\times 200$.

FIG. 4. Hemorrhagic encephalitis showing rupture of a small vessel with erythrocytes that have apparently passed out into the perivascular space. Note the sharp limitation of the hemorrhage to the perivascular space with only slight involvement of the surrounding brain tissue. Hematoxylin and eosin stain. $\times 400$.

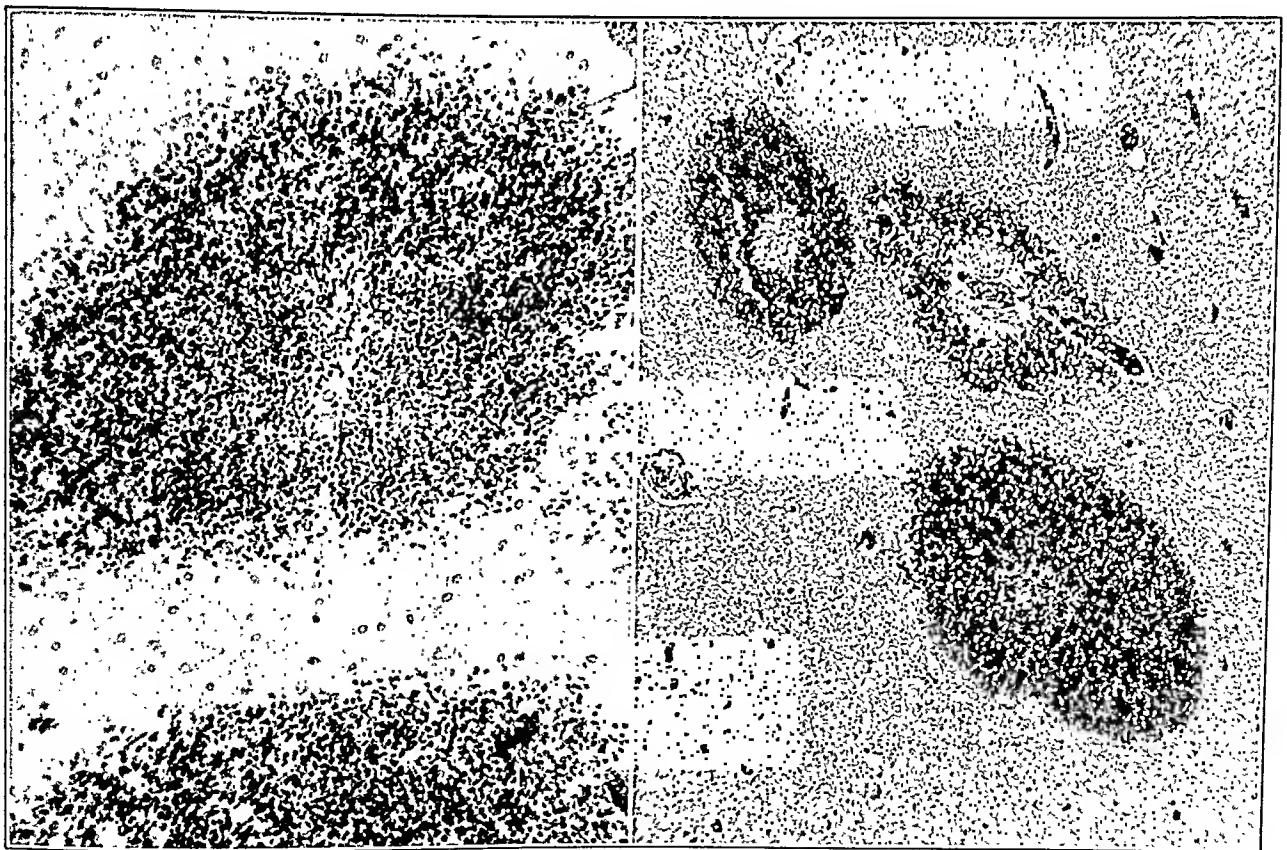
FIG. 5. A "ball" or solid hemorrhage from a case of hemorrhagic encephalitis. Although no vessel is visible in this hemorrhage, the rounded uniform arrangement suggests a vascular origin. Hematoxylin and eosin stain. $\times 200$.

FIG. 6. Hemorrhagic encephalitis showing ring hemorrhages. Note the clear uninvolved center surrounded by a dense ring of erythrocytes. These hemorrhages also have a tendency to remain discrete. They are caused by rupture of the numerous capillaries that surround the larger vessels. Azocarmine stain. $\times 75$.



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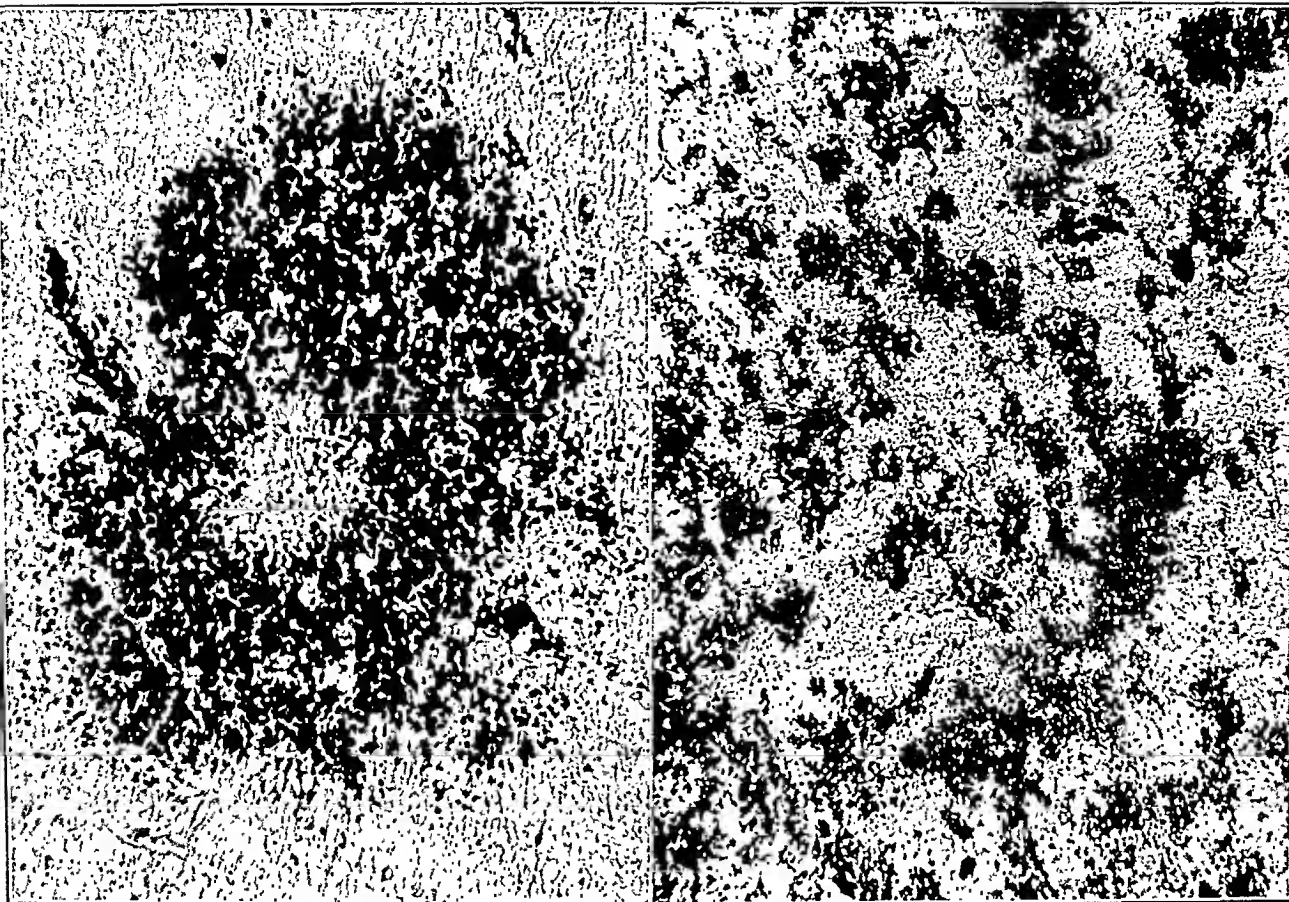


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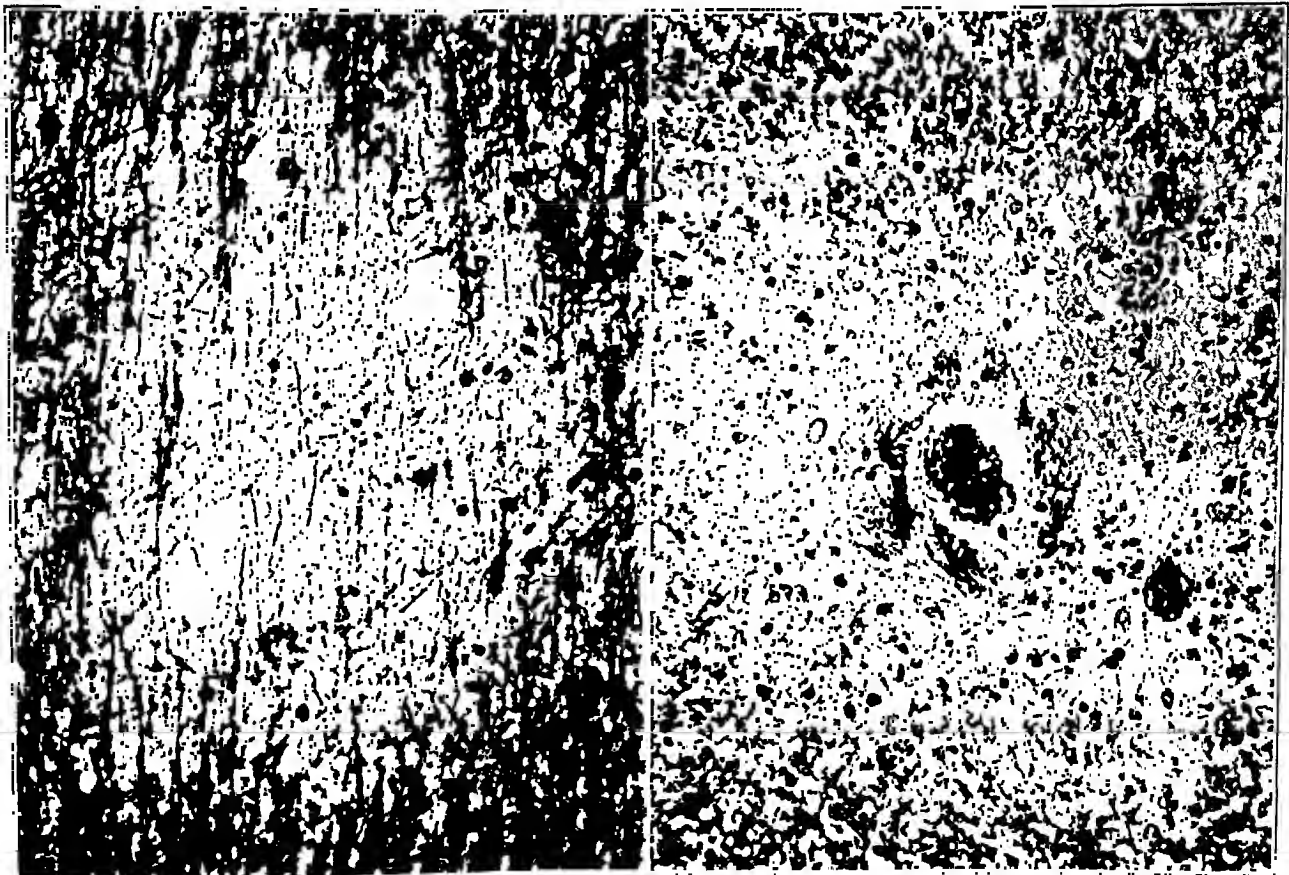
PLATE 28

- FIG. 7. Hemorrhagic encephalitis, showing a ring hemorrhage under higher magnification. Note the tendency of the red cells to remain localized. Azocarmine stain. $\times 200$.
- FIG. 8. A case of hemorrhagic encephalitis, in which the hemorrhages are diffuse and not limited to the region of the larger blood vessels. These petechiae are small and therefore have caused but slight injury to the surrounding brain tissue. Azocarmine stain. $\times 200$.
- FIG. 9. Hemorrhagic encephalitis, showing the thinning out and rupture of numerous brain fibers by a large hemorrhage. Note that an occasional fiber is still intact and passes through the mass of erythrocytes. The invasion of the scavenger cells cannot be seen with this stain. Modified Laidlaw silver stain. $\times 400$.
- FIG. 10. Hemorrhagic encephalitis showing complete demyelination about a cerebral vessel, from a patient who died 6 days after the onset of illness. Note the relative absence of scavenger cells and erythrocytes. There is an increase of glial fibers laid down by the proliferating astrocytes. Iron hematoxylin - Van Gieson stain. $\times 200$.



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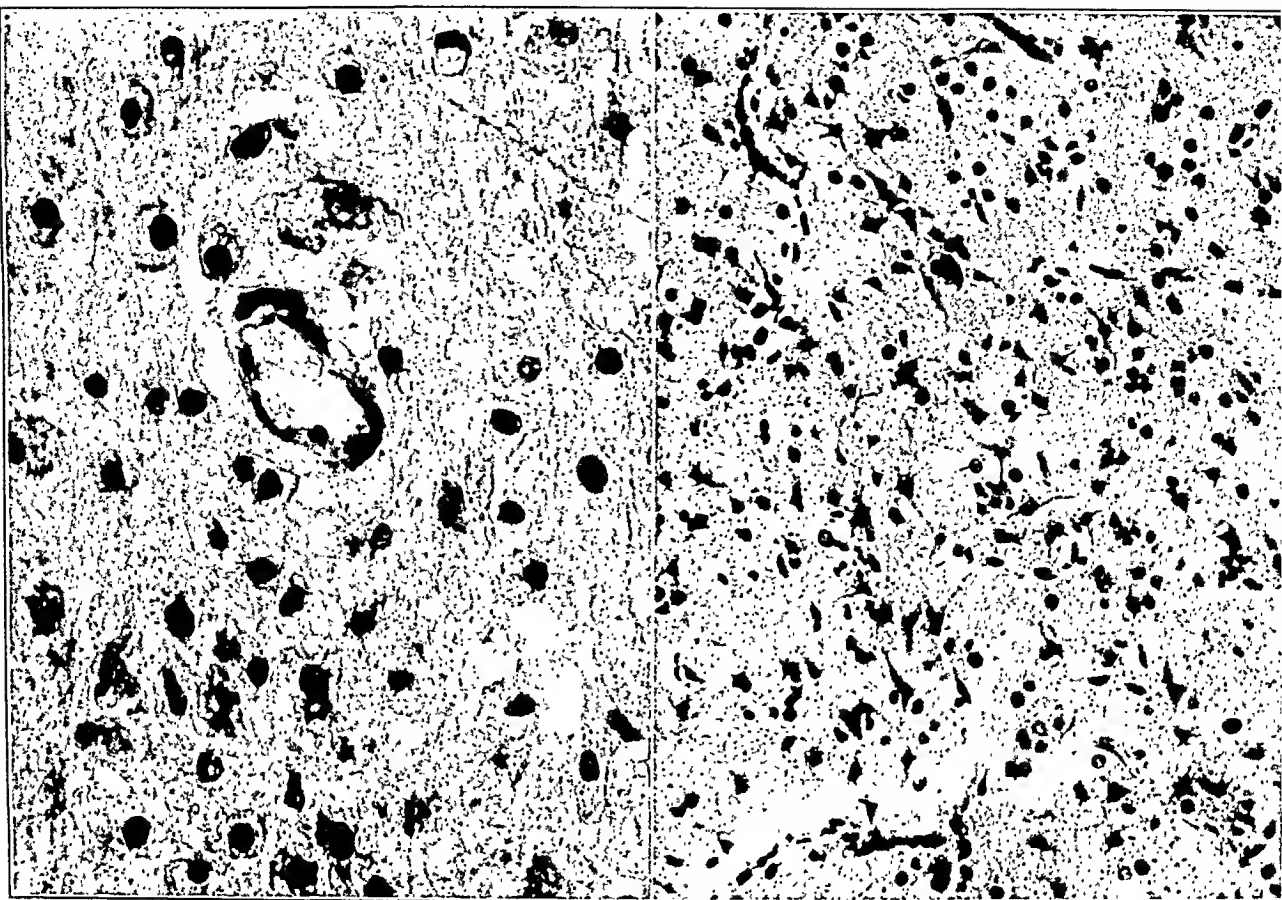


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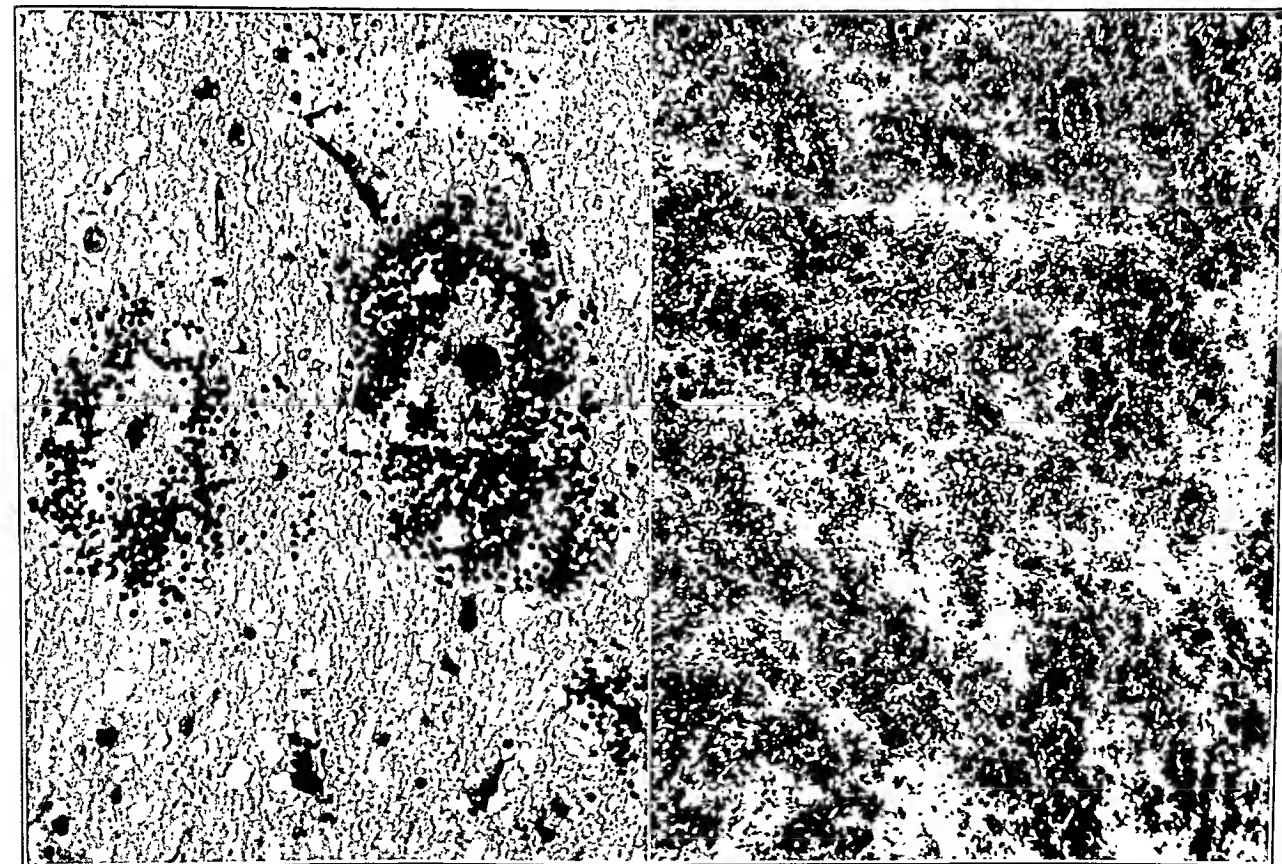
PLATE 29

- FIG. 11. Hemorrhagic encephalitis, early perivascular demyelination. Note the beginning infiltration of fat-granule cells. There are no free erythrocytes surrounding the vessel. Hematoxylin and eosin stain. $\times 400$.
- FIG. 12. Hemorrhagic encephalitis showing a marked increase of microglia in an area of cerebral injury. Many of the cells have just arrived and still possess their wavy branched processes. Others have already changed into "rod cells." No fat-granule cells have, as yet, been formed. Del Río-Hortega's silver carbonate stain. $\times 400$.
- FIG. 13. Petechiae in the brain of a patient dead of arsenic poisoning. The hemorrhages are mostly of the ring type. Thrombi are present in the involved vessels. No tissue destruction is apparent. Azocarmine stain. $\times 400$.
- FIG. 14. Diffuse extravasations of red cells throughout the brain tissue in a case of purpura. There appears to be no definite localization of the erythrocytes about larger vessels. Azocarmine stain. $\times 200$.



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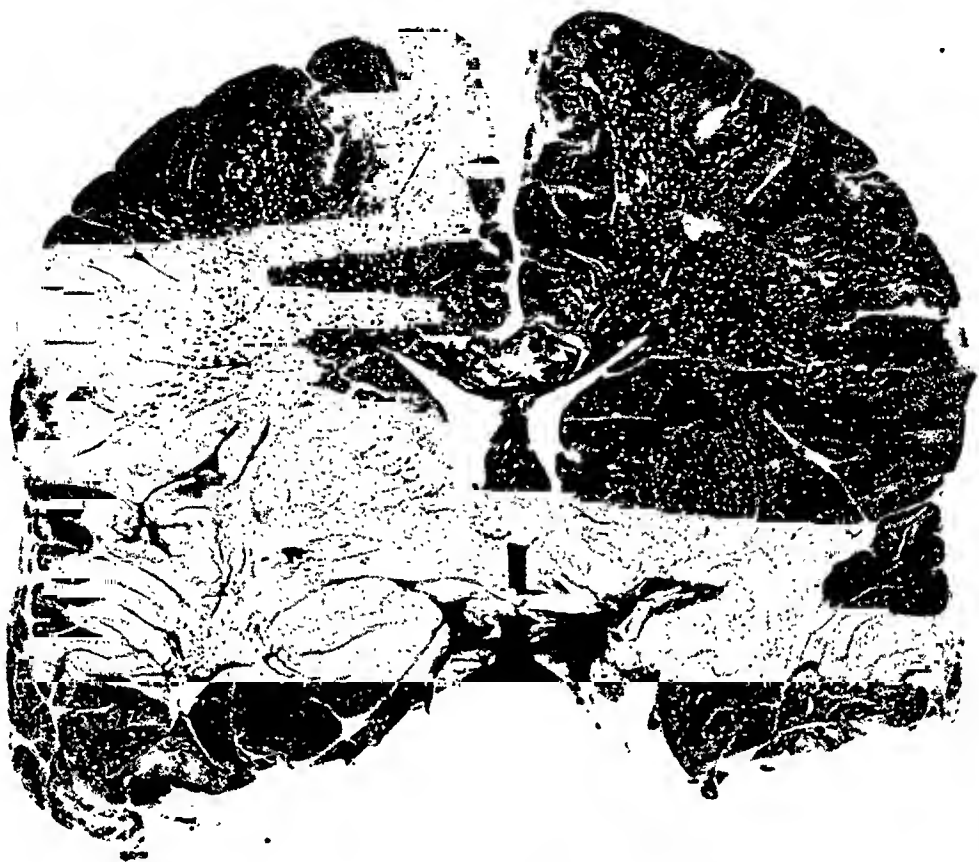
Baker

Hemorrhagic Encephalitis

PLATE 30

FIG. 15. Coronal section through the brain of a patient dead of a bilateral bronchopneumonia. The cerebral tissue is filled with small discrete hemorrhages which tend to be limited to the white substance. The basal nuclei are relatively uninvolved. No gross areas of cerebral softening are visible. $\times 0.8$.

FIG. 16. Traumatic cerebral hemorrhages. Most of the petechiae are found in the white matter. Note the small bruised area in cortex of the brain at the margin of the sagittal suture on the right. There is a tendency for most of the hemorrhages to arrange themselves in tiny groups instead of being disseminated throughout the tissue. $\times 0.8$.



15



16

DISTRIBUTION OF NUCLEAR INCLUSIONS IN WILD ANIMALS *

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The viruses that are most destructive receive the closest attention. But biologically the less pathogenic ones, the presence of which is not revealed by distinctive clinical symptoms, are equally important to those who wish to understand the place in nature of these elusive organisms or substances. The discovery of the latter has been largely a matter of chance. In 1920 Jackson¹ reported intranuclear inclusions, which she called protozoan parasites, in the salivary glands of guinea pigs, explaining that they were observed in examinations made for an entirely different purpose. We now know that they are caused by a virus. During the routine study of the tissues of rats in a large dietary experiment, Thompson² discovered somewhat similar inclusions also in the salivary glands. In the course of investigations on Rift Valley fever, Findlay³ found intranuclear inclusions in the liver cells of mice which showed no symptoms of disease and were caused by a hitherto unrecognized mouse virus. Many other instances of chance favoring the prepared mind might be cited.

If we try to plot out the distribution of nuclear inclusions which have been proved experimentally to be of virus etiology and others under suspicion in the vertebrate series, we find them in 2 fish, 1 amphibian, no reptiles, 4 birds and at least 11 mammals. This list includes only the species in some individuals of which they naturally occur. It does not include a considerable number of other species in which inclusion formation can be induced by injection of viruses foreign to them. Impressed by the need for more data on the distribution of intranuclear inclusions, and possibly of viruses, particularly in wild animals, the Rectors⁴ made a short survey of the tissues of such species in the vicinity of St. Louis and found a remarkable case of their development, without symptoms, in the salivary glands of common moles. The purpose of this paper is to report briefly a

* Aided by grants from the Rockefeller Foundation to Washington University for research in science and in virus diseases.

Received for publication August 24, 1934.

more detailed survey chiefly of the kidneys of mammals and birds from the pathological service of the Zoological Society of Philadelphia.

MATERIAL

This included 165 animals of which 78 were mammals and 87 were birds. The mammals belonged to 58 species distributed as follows: *Artiodactyla* 16, *Primates* 10, *Xenarthra* 1, *Rodentia* 8, *Carnivora* 21 and *Marsupialia* 2. The birds comprised 62 species as follows: *Passeriformes* 5, *Strigiformes* 2, *Coraciiformes* 1, *Piciformes* 1, *Psittaciformes* 20, *Columbiformes* 2, *Ralliformes* 3, *Galliformes* 4, *Accipitriformes* 9, *Anseriformes* 8, *Ardeiformes* 4, *Struthioniformes* 1, *Baleariciformes* 1 and *Phoenicopteriformes* 1. The majority, negative as far as inclusions are concerned, are listed later by themselves and the few positive are given in Table I.

Most of the tissues were fixed in formalin or Zenker's fluid and sections were colored routinely with hematoxylin and eosin. In special cases Giemsa's stain was also employed. By reference to the number accompanying each specimen complete clinical and pathological reports could be secured from the records of the Zoological Society. A large collection of specimens showing nuclear inclusions in virus diseases was available for comparison in the Anatomical Laboratory of Washington University.

OBSERVATIONS

Search was made for the type A and B inclusions of Cowdry.⁵ Under type A are grouped those caused by the viruses of herpes, yellow fever, mad itch, fox encephalitis and so on; and under type B those in Borna disease, poliomyelitis, and so on. In both there is an increase in acidophilic material in the nucleus but there the similarity ends. Type A inclusions are formed by the accumulation in the nucleus of granular acidophilic material and the margination on the nuclear membrane of all the basophilic nuclear substance. At a certain stage in their development a clear space is interposed between the central, acidophilic and the peripheral basophilic materials. The nucleoli are altered, move to the nuclear membrane, disappear and the inclusion-laden nuclei disintegrate.

Type B inclusions, on the contrary, are usually spherical, often hyaline, droplet-like bodies which make their appearance in nuclei

that otherwise look fairly normal. The basophilic chromatin is not margined in the same way but when the inclusions become very large, as they sometimes do, it is pushed away toward the nuclear membrane. The nucleoli persist for a longer time and nuclear disintegration is less marked.

To recognize well developed inclusions of either type is not difficult. It is the border line cases that give trouble. Normally the nuclei of the renal tubules of mammals and birds are rounded bodies of fairly uniform size. One, occasionally more, nucleoli are present and are the only nuclear structures visible in them in the living unstained state. But it is with fixed tissues that we have to deal. In them the nucleoli generally color with basic dyes, but they are really amphinucleoli, for they contain some acidophilic as well as basophilic material. The association of the two components is not always the same. It is when the basophilic material is mixed with the acidophilic that the nucleolus as a whole colors blue with Giemsa or hematoxylin and eosin. Frequently the basophilic component is applied rather unevenly on the surface of acidophilic substance, which is more definitely spherical in form and acts as a kind of core (Figs. 2 and 16). More rarely it becomes detached altogether from the acidophilic mass, in which case the latter is commonly called an acidophilic nucleolus, or plasmasome, but this is not illustrated in our figures. The prefix, plasma, indicates something staining with plasmatic, acid dyes in contrast to nuclear, basic ones. In addition to the nucleolus the nuclei contain irregular masses and strands of basophilic material which are generally more marked as the basophilic nuclear membrane is approached. A small amount of acidophilic material can also be distinguished in the nucleoplasm. Sometimes it appears as a thin, more or less homogeneous deposit in which condition it is represented in gray near the nucleoli in Figures 1 and 2. It may, however, appear in the form of fairly discrete particles like those which make up inclusions caused by viruses. Its relation to the acidophilic nuclear core is not known. Microincineration shows that the latter is much richer in mineral constituents, but this difference may be only one of degree conditioned by the greater density of the nuclear core. In the nuclei of the convoluted tubules the extranucleolar acidophilic material and the nucleoli are more noticeable than in the nuclei of the collecting tubules. In the birds' kidneys they are slightly more prominent than in mammals.

But the tissues examined were not normal. They came from an extensive autopsy service and the nuclei exhibited a wide variety of modifications. We shall eliminate first those that we do not consider to have any definite bearing on our problem.

Increases in acidophilic material to the degree illustrated in gray in Figures 1 and 2 were listed as negative. As was inevitable, some of the tissues showed postmortem autolysis. In such cases the acidophilic nuclear material referred to becomes more prominent (Fig. 5) and other changes occur which will be described in detail by Lucas and Cowdry⁶; but the alteration sweeps through the tissue fairly uniformly in the case of the epithelial cells present and for this reason can easily be distinguished from the effect of virus action which is more localized, and generally, but not always, accompanied by characteristic lesions.

Hypertrophy of individual nuclei (Fig. 18) and of groups of nuclei was very commonly seen without either type A or B inclusions. It was observed in 18 species of birds and was most marked in a South American porcupine. In 5 mammalian species and in 1 bird this was accompanied by margination of basophilic chromatin. Margination alone without increase in central acidophilic material (Fig. 6) was observed in 9 mammalian species and 4 avian ones. Sometimes the nucleoli were enlarged (Fig. 16).

Though alterations such as these were noted in some of the following specimens, no definite intranuclear inclusions suggestive at all of virus action were detected. The specimens are listed because any study of distribution must take into consideration negative findings. Only the kidneys were examined, unless other organs are mentioned. The numerals in brackets indicate the number of individuals of a given species studied when this exceeded one.

MAMMALS

<i>Artiodactyla</i>	Western water buck
Klipspringer	Apis deer
Llama	Japanese sika deer
Indian buffalo	Hog deer
Blesbok	Central American deer (2)
Himalayan thar	Virginia deer
White tailed gnu	Red deer
Bison (3)	Pigmy hippopotamus
American elk	

Primates

Green monkey (2)
 Kra macaque
 Orang utan
 Hocheur monkey
 Weeper cebus (2)
 Common marmoset
 Silky marmoset

Himalayan bear
 European brown bear
 Black bear
 European badger
 Mink (2)
 Polecat
 Otter
 White nosed coati (2)
 Ring tailed coati

Xenarthra

Long haired armadillo

Crab eating raccoon
 Raccoon (3)

Rodentia

Canada porcupine (3)
 Porcupine
 Capybara
 Coypu rat
 Prairie dog
 Prevost's squirrel
 Gray squirrel (2)

Coyote
 Red fox
 Carpathian fox
 Jackal
 Two spotted paradoxure
 African civet
 Wild cat
 Caracal lynx
 Eyra cat (2)

Carnivora

Sea lion

Marsupialia

Common opossum (6)
 Red kangaroo

BIRDS

Passeriformes

Canary
 American robin
 European jay
 Wild grackle (brain only)
 Common crow (2)

Salles amazon
 White fronted amazon (2)
 (also liver and lung)
 Yellow fronted amazon (3)
 (also liver and lung)

Strigiformes

Barn owl
 Spectacled owl

Salvin's amazon
 Green cheeked amazon (2)
 (also liver)

Coraciiformes

Abyssinian ground hornbill

Golden naped amazon
 Festive amazon
 Levaillant's amazon (7)
 (also liver and lung)

Piciformes

Cuvier's toucan

Wild parrots (6)
 (also brain)

Psittaciformes

Undulated grass parrakeet
 Parrakeet
 Blue and yellow macaw (liver only)
 Red and blue macaw
 Red sided eclectus

Red vented parrot
 St. Vincent's parrot
 Lesser sulphur crested cockatoo
 Sulphur crested cockatoo

Columbiformes

Barbary turtle dove

<i>Ralliformes</i>	<i>Anseriformes</i>
Common coot	Gargany teal
Purple gallinule	Muscovy duck
Florida gallinule	Mallard duck (2)
	Lesser white fronted goose
<i>Galliformes</i>	Hybrid goose
Common peafowl (2)	Egyptian goose
Rain quail	
Vulturine guinea fowl	<i>Ardeiformes</i>
	White necked stork
<i>Accipitriformes</i>	White stork
Ruppel's vulture	Scarlet ibis (2)
Abyssinian vulture	Straw necked ibis
Cooper's hawk (2)	
Red shouldered hawk	<i>Struthioniformes</i>
Goshawk	Somali ostrich (2)
Marsh hawk	
Red tailed hawk (2)	<i>Balcariciformes</i>
Battleur eagle	Demoiselle crane
Bald eagle	<i>Phoenicopteriformes</i>
	Ruddy flamingo

The intranuclear inclusions that we desire to report are listed in Table I.

Type A inclusions, found in the kidneys of two Guatemalan amazons (Figs. 9-15), were interesting because they exhibited points of resemblance to the inclusions caused by the yellow fever virus. Compare Figures 9 and 10 with Cowdry and Kitchen's ⁷ Figure 16, and Figure 11 with their Figure 24. In both, the nuclear inclusions are made up of sharply outlined particles of fairly uniform size. They appear in the form of clumps in the zone intermediate between the nucleolus and nuclear membrane. But in the Guatemalan amazon fewer nuclei show them and the condition did not lead to necrosis. In amazon No. 10602 there were approximately 1.164 inclusion-laden nuclei per oil immersion field and in No. 10240, 0.125. The sections were about 8 microns thick. A simple calculation gives the incidence of inclusions in the sections more accurately, in the first at 65.6 per sq. mm. and in the second at 7.07. In addition to the kidneys the liver, lung, testis, epididymis, striated and heart muscle of amazon No. 10602 and the liver, lung, testis, epididymis, pancreas, spleen and intestine of amazon No. 10240 were examined without finding any more type A inclusions. Type B were, however, found abundantly in the lungs of No. 10602 (Figs. 14 and 15), but were absent in the lungs of No. 10240, despite the presence in both of similar

lesions of advanced anthracosis. Nothing in the history of either animal suggests a virus infection. The autopsy reports were:

No. 10602: Injury and malnutrition; edema, congestion, hemor-

TABLE I

Animal	Type A inclusions	Type B inclusions	Figures
<i>Mammals</i>			
<i>Artiodactyla</i> — Red deer		+	
<i>Primates</i> — Mongoose lemur		+	3
“ — Kra macaque		+	
“ — Rhesus macaque		+	4
“ — Lion tailed macaque		+	
<i>Rodentia</i> — South American porcupine			
<i>Birds</i>			
<i>Piciformes</i> — Cuvier's toucan (liver, lung, pancreas, intestine, spleen — negative)		+	7 and 8
<i>Psittaciformes</i> — Guatemalan amazon, 10,240 (liver, lung, pancreas, intestine, testis, epididymis, and spleen — negative)	+		9 and 10
“ — Guatemalan amazon, 10,602 lung (liver, striated and cardiac muscle, testis and epididymis — negative)	+	+	11, 12 and 13
“ — Black crested cockatoo	+?		17
<i>Columbiformes</i> — Wonga Wonga pigeon		+	18
<i>Galliformes</i> — Silver pheasant		+	
<i>Accipitriformes</i> — Marsh hawk		+	20
<i>Anseriformes</i> — Ceropsis goose		+	21
“ — Spur winged goose		+	

rhage and anthracosis of lungs; myocardial degeneration and chronic endocarditis (?), atrophy of spleen and thyroid; atrophy and fibrosis of adrenal; pigmentation and hydropic degeneration of liver; atro-

phic enteritis, atherosclerosis (carotid and aorta); hyperplasia of bone marrow.

No. 10240: Pigmentation, passive congestion and multiple abscesses of liver (Gram-negative bacilli in smears); hyperplasia of bone marrow; acute necrotizing enteritis; hydropic degeneration of pancreas; cloudy swelling of kidney; edema, congestion and abscess formation of lungs; pigmentation, diffuse hyperplasia and myeloid metaplasia of spleen.

The nuclear inclusions in the black crested cockatoo were less noticeable and it is doubtful whether they merit inclusion in type A, although the nucleus illustrated in Figure 17 could not itself be distinguished from one affected by the virus of herpes for example. It is the infrequency of such nuclei, coupled with the absence of tissue reaction and the unsatisfactory way that the tissue stained, which makes the diagnosis doubtful.

Nuclei containing type B inclusions were spread quite widely in the preparations among other nuclei which showed no inclusions; but in the marsh hawk the change was sharply localized, being limited to the nuclei of a single collecting tubule (Fig. 20). These type B inclusions are clearly of more frequent occurrence than those of type A and may (Figs. 4, 8 and 20) or may not (Figs. 3, 7, 14, 15 and 21) be accompanied by nuclear hypertrophy; whereas the type A inclusions in the amazon and cockatoo did not make their appearance in particularly enlarged nuclei; the largest is represented in Figure 13. No characteristic lesions, either infiltrative, proliferative or degenerative, were found with the type B inclusions, neither did the clinical history or the autopsy findings give any reason to believe that a virus was operative. Yet some of these type B inclusions were as large and conspicuous as any reported in the literature. We compared them particularly with inclusions in the kidneys of sewer rats, of which specimens were very kindly sent to us by Dr. E. Hindle.⁸

The crystalline inclusion represented in Figure 19 was unique. Others like it were not found even in the same preparation. It is quite different in appearance from the large intranuclear acidophilic crystals first reported by Szymonowicz and Macallum and found also by Cowdry and Scott⁹ in the livers of dogs.

Before attempting to discuss the possible significance of these observations we wish to report the results of a search for nuclear

inclusions in kidney specimens from 1012 human autopsies courteously loaned to us by Dr. H. Gideon Wells of Chicago. No type A inclusions were found. Type B inclusions were seen in only 17 kidneys, or 1.67 per cent, as contrasted with 14 out of 163 animals, or 8.58 per cent.

But the incidence of nuclear inclusions may not be so much lower in the human series than in the wild animals as the figures indicate. If the search for them in the humans had been equally thorough a few more might have been found. The sections of the human kidneys had all been well stained with hematoxylin and eosin, which is the most satisfactory method for inclusions, because of color contrast and permanency, but some of them were rather too thick. It is more difficult to identify inclusions by focussing up and down through entire nuclei than in preparations about 5 microns thick in which many nuclei are cut in section. But none of the sections were so thick that they could not be easily studied with a 3 mm. oil immersion objective. Postmortem changes were rather more frequent in the human series. In such cases, the shrinkage of the nuclei and alteration in staining reaction would have interfered with the identification of inclusions, particularly those of type A, if not well developed.

On the other hand pathological lesions were much more numerous and varied in the human than in the wild animal series. With greater frequency in functional disturbance, one would expect a somewhat higher incidence of inclusion formation quite irrespective of whether they are caused by viruses or cellular environments unusual in osmotic or other features. Indeed, the remarkable rarity of nuclear inclusions in the human series shows that, in the kidney at any rate, they are not ordinary, commonly encountered nuclear degenerations. Comparison of the 14 kidneys, in which they were seen, with the others failed to reveal any distinctive pathological process. None of the inclusions were so large and noticeable as those observed in some of the wild animals and others in preparations of frog and rat kidneys kindly sent to us by Dr. B. Lucké and Dr. E. Hindle respectively (see Lucké,¹⁰ Figs. 14 and 15, and Hindle⁸). Neither were they so conspicuous as the inclusions reported in the kidneys of fetuses and young children by many authors (see Farber and Wolbach¹¹). The kidneys that we examined were almost entirely from adults. It is possible that such inclusions, though present

earlier, had disappeared, or that a geographic factor of some kind operates, as suggested by Farber and Wolbach, and that for some unknown reason this factor is less widespread in the Middle West.

DISCUSSION

This examination of many specimens of the kidneys of wild animals and humans has been a fatiguing but very profitable experience. It has not brought to light a single instance of inclusions that we care to attribute definitely to virus action. Those which most nearly qualify were the A inclusions in the Guatemalan amazon and the B inclusions in the marsh hawk. Neither was accompanied by an infiltrative tissue reaction. It is clear that type A are less common nuclear modifications than type B. How are we to explain their formation?

Obviously about all we can do is to mention some of the possible factors. Wells¹² states that "it is probable that chemically the differences between nucleus and cytoplasm are quantitative rather than qualitative." This view is supported by recent studies on microincineration which show that nuclei are richer in mineral constituents than was formerly supposed (Scott¹³). Chromatin holds our attention because it stains vividly and on account of its great physiological significance, but it is one of many nuclear components. Chemically, chromatin is a protein salt of nucleic acid. According to Mathews,¹⁴ "the nucleic acid is apparently the same, or at any rate closely similar in all the different cells examined; but the protein base appears to be characteristic of the cell." The firm, jelly-like consistence of most nuclei he attributes to the gelatinizing property of solutions of nucleic acid salts. The nucleic acid is basophilic for it combines with "basic" dyes. That "basic chromatin" carries a negative electrical charge has been shown by McClendon.¹⁵ When cells are placed in an electric current he observed the actual pushing out of the nuclear membrane toward the anode by pressure of the basic chromatin. Acidophilic material carries, on the contrary, a positive charge. The outstanding feature of the formation of type A inclusions is a change involving the entire nucleus by which the acidophilic material, perhaps the protein, protamin or histon base of the nucleic acid salt, becomes separated from the basophilic nucleic acid and accumulates in the center, whereas the acid moves to the

periphery of the nucleus. But the acidophilic substance may be only partly or not at all of chromatinic origin. Other nuclear components than chromatin may be involved and there is a possibility that some of it may enter the nucleus from the surrounding cytoplasm, for there is usually some hypertrophy which must be due to intake of extraneous material, though it may simply be water.

At first sight one gains the impression that an alteration of this sort might result from the action of a nuclease. Van Herwerden¹⁶ was able to remove selectively basophilic components from several types of cells by allowing a nuclease to act on sections of alcohol, or hot water, fixed tissue. We experimented with a nuclease kindly prepared for us by Dr. D. J. Kooyman. This we injected into the living epidermis, because we thought that in an avascular situation we might secure a maximum change. But stained sections failed to show any sign of the development of type A inclusions. Moreover, in our studies on postmortem autolysis chromatin is broken down, presumably by nuclease, but inclusions are not formed. Lee,¹⁷ working in this laboratory, has been more nearly successful in his investigation of osmotic factors producing acidophilic inclusions which very closely resemble those caused by viruses. The process, whatever it is, of type A inclusion formation ordinarily goes on to complete nuclear disintegration and cellular necrosis. It is generally accompanied by a local invasion of phagocytes.

Type B inclusions occurred more frequently, both in the wild animals and humans. The chromatin is not divided in quite the same way into central acidophilic and marginating basophilic fractions. When they are small the nuclear structure about them is not modified, except for the appearance of a delicate halo. When they increase greatly in size the contents of the nucleus are crowded toward the nuclear membrane. As with beginning type A inclusions, it is not possible to draw a definite line of distinction between the inclusions themselves and acidophilic material normally present in small quantities in the nuclei. The nuclear change is less radical than in type A. It has the appearance of developing more slowly and the terminal stage of necrosis is seldom seen. This may explain why type B inclusions often occur without an associated tissue reaction, toxic substances perhaps not being liberated to the same degree as in the development of A inclusions. We do not deny that type B inclusions may be the result of virus action but we think that they

may be caused directly by other factors as well, or secondarily by viruses activating or influencing these other factors. In estimating the probability that any given type B inclusions are due to virus action several considerations are important.

The first is the extent of deviation from normal. When the inclusions are of small size, consisting only of droplets of acidophilic material in nuclei whose cells otherwise look as usual, one is inclined to dismiss them as of little if any significance from the point of view of viruses. Another feature that leads to scepticism is uniformity in distribution of the inclusions in practically all cells of the same sort in a preparation; for, as already stated, the action of viruses is generally limited to relatively small foci and unequal in degree, some cells being altered slightly and others more severely, as if they had been attacked one after another.

Inclusions of this type B are only exceptionally marked by a tissue reaction detectable by routine techniques, by which we mean necrosis of cells, infiltration by phagocytes, fibrosis and so on. It is possible that the application of special cytological and microchemical methods might reveal alterations in the neighboring epithelial cells and we exclude arbitrarily, in specifying the tissue reaction, hypertrophy and other modifications in the inclusion-laden cells themselves. A tissue reaction of this kind was not a noticeable accompaniment of type B inclusions in either our wild animal or human series. It may have occurred to a mild degree and have disappeared, or the inclusion formation in any particular locality may perhaps be secondary to considerable increase in virus elsewhere and transport of the virus to the area by the blood stream. This may apply also to certain type A inclusions. A dog which had been inoculated with fox encephalitis virus showed in its kidney (given to us by Dr. R. G. Green ¹⁶) inclusions limited to the renal glomeruli and there was no tissue reaction. But the affinity of this virus is primarily for endothelium. Moreover, the inclusions in fetuses and young children are often devoid of tissue reaction, as noted by Farber and Wolbach.¹¹

Early type B inclusions may be of the same size as nucleoli or considerably smaller. One or more of the following distinguishing features can generally be made out. (1) Clear halos common about the inclusions are rare or absent about the nucleoli. (2) The inclusions are not definitely placed in the nuclei and of limited number like the nucleoli. (3) When several inclusions occur in a single nu-

cleus, they often exhibit a gradation in size from bodies much smaller than nucleoli to considerably larger ones. (4) They are usually wholly acidophilic, whereas the nucleoli may color with both acid and basic dyes or consist of an acidophilic core on which basophilic material is plastered.

Further investigations may bring to light other differences. Nucleoli differ from type A inclusions in the possession of large amounts of mineral.^{19, 20} We have incinerated the type B inclusions in Hindle's sections of rat's kidneys and wish to report very little mineral, but the specimens were not fixed by the best method for microincineration. We have no data for other type B inclusions but we think it likely that this difference from nucleoli will hold. Nucleoli are known to be very compact structures. In the nerve cells of some lower forms they settle in the lowest parts of the nuclei by their own weight and most histologists have seen instances of nucleoli carried right out of the nuclei by the microtome knife. A careful comparison of both types of inclusions with nucleoli and basophilic chromatin in centrifuged cells is indicated.

When, on the other hand, type B inclusions are localized in distribution, are very large, and the containing nuclei are much distended, we entertain the possibility of virus action and await experimental proof. This is the status of the inclusions in the kidney of the marsh hawk and in the kidneys of sewer rats of Hindle. Bodies difficult to distinguish morphologically from inclusions of type B apparently occur normally in the cells of the vesicula seminalis of certain mammals and humans,²¹⁻²⁴ and in those of the nucleus supraopticus and paraventricularis of the midbrain of many vertebrates and humans.²⁵⁻²⁸ In both localities they are interpreted as phases in the elaboration of nuclear secretions, for which, however, the evidence, in our opinion, is insufficient. But their similarity to inclusions in other tissues, which some have attributed to virus action, inspires caution.

How type B inclusions are formed remains to be determined. Splitting of the nucleic acid salt of protein is not a conspicuous feature. When first they begin to grow, the internal organization of the nuclei is not lost. They seem to be passive accumulations of substance, the nature of which awaits chemical analysis. All we know at present is that, like the type A inclusions, they contain little or no thymonucleic acid and are not of fatty nature. They may conceivably be produced by faulty elimination of nuclear material or by the entry of extraneous material in unusual amounts. Either might be

occasioned by a modification in the permeability of the nuclear membrane, which in turn might result directly or indirectly from the operation of a virus or simply from a functional derangement of common occurrence in some tissues and very rarely seen in others, depending upon their intrinsic attributes.

SUMMARY

Type A inclusions resemble those of herpes and usually: (1) exhibit a change which sweeps through the entire nucleus whereby acidophilic material accumulates in the center to form the inclusion and basophilic material marginates on the nuclear membrane; (2) lead to complete disintegration of the injured cells, and (3) are accompanied by a noticeable tissue reaction. Inclusions of this kind were seen only in the kidneys of 2 Guatemalan amazons. In so far that each inclusion was made up of clumps of discrete acidophilic particles and the nucleoli were not displaced early in the process, they look quite like the intranuclear inclusions caused by the yellow fever virus, but they were rather evenly distributed in the tissue and were not accompanied by distinctive lesions.

Type B inclusions resemble those in Borna disease and usually: (1) develop as local accumulations of acidophilic material in nuclei, the remaining parts of which at first show no other modifications; (2) crowd all other nuclear materials toward the nuclear membrane as they increase in size; (3) do not lead to extensive nuclear disintegration or to severe accompanying tissue reactions. These were more widely distributed. They were found in the lungs of one of the Guatemalan amazons and in the kidneys of 7 out of 62 species of birds, 6 out of 58 species of mammals and 17 out of 1012 individual humans.

Acidophilic nuclear material is normally present in small amounts in the nuclei of renal epithelial cells. There is no sharp line of distinction between it and inclusions of either type. It is the degree of change that raises the question of virus etiology. When the inclusions are (1) so conspicuous that they can be seen without the aid of oil immersion objectives, (2) localized in distribution, and (3) related to marked lesions, one looks for a virus. But no type A inclusions, and especially no type B ones, which are so much more common, should be regarded as pathognomonic of virus action without experimental proof.

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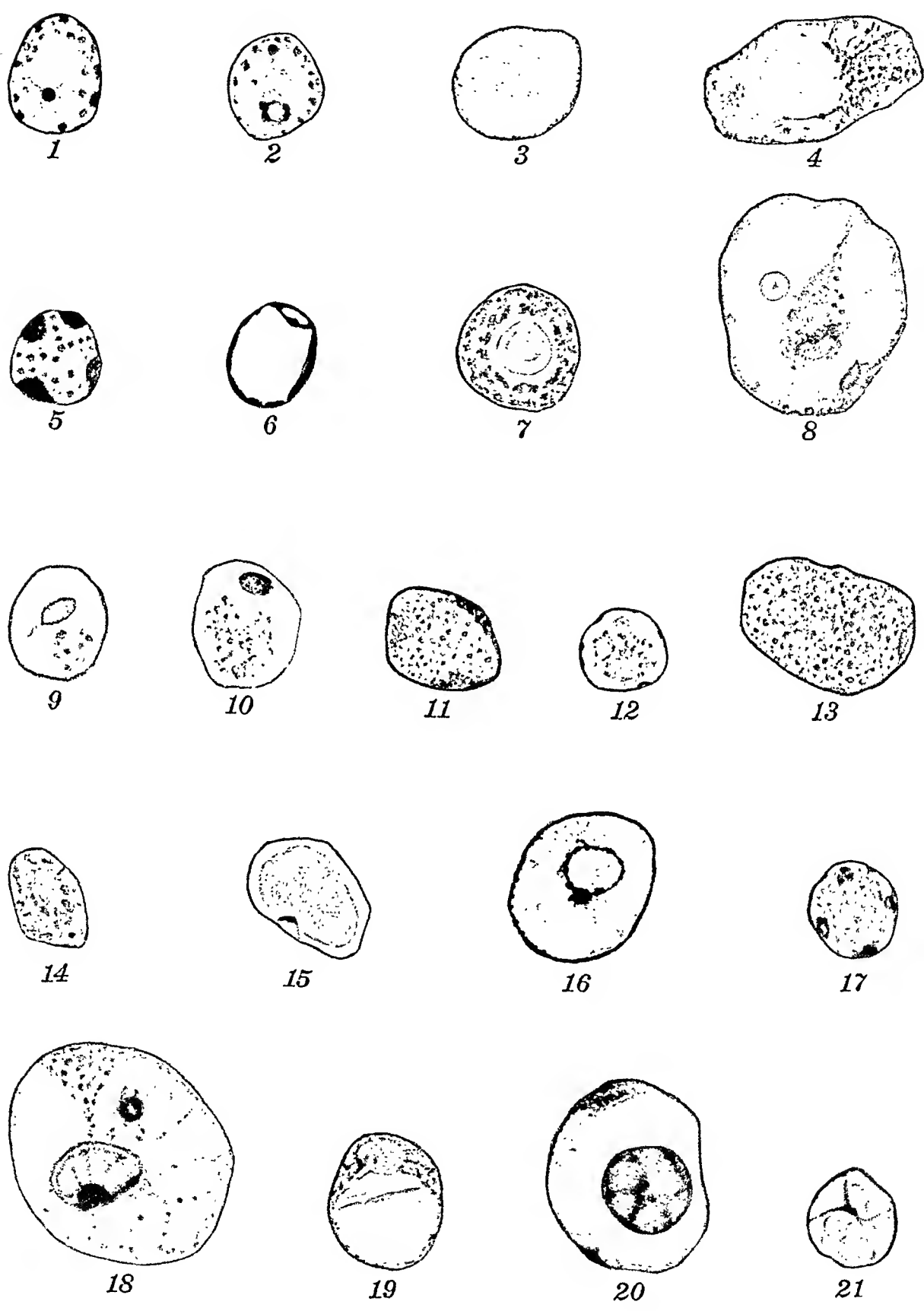
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DESCRIPTION OF PLATE

PLATE 31

The drawings of selected nuclei were made with camera lucida at a magnification of 2400 diameters. Nuclear material, which was acidophilic and colored red in the original stained preparations, is represented in shades of gray and basophilic material, which was blue, in black or very dark gray.

- FIG. 1. *Canada porcupine*. Convoluted tubule. A little acidophilic material is shown near the nucleolus.
- FIG. 2. *Straw necked ibis*. Convoluted tubule. Slightly more acidophilic material. Nucleolus with an acidophilic core.
- FIG. 3. *Mongoose lemur*. Convoluted tubule. Type B inclusion.
- FIG. 4. *Rhesus macaque*. Convoluted tubule. Type B inclusion; basophilic chromatin not margined, just pushed aside; nuclear hypertrophy.
- FIG. 5. *Prairie dog*. Convoluted tubule. Marginal clumping of chromatin with central basophilic granules.
- FIG. 6. *Red kangaroo*. Convoluted tubule. Margination of basophilic chromatin alone without inclusion formation.
- FIG. 7. *Cuvier's toucan*. Convoluted tubule. Type B inclusion surrounded by a distinct halo.
- FIG. 8. *Cuvier's toucan*. Convoluted tubule. Small type B inclusion with marked nuclear hypertrophy.
- FIGS. 9-10. *Guatemalan amazon*. (No. 10240). Convoluted tubule. Two stages in development of type A inclusions leading to complete margination of basophilic chromatin.
- FIGS. 11-13. *Guatemalan amazon* (No. 10602). Convoluted tubule. A further stage in inclusion formation (11). Coalescence of acidophilic inclusion particles in a shrunken and a hypertrophied nucleus (12 and 13).
- FIGS. 14 and 15. Same. Type B inclusions in epithelial cells of the lung.
- FIG. 16. *Yellow fronted amazon*. Convoluted tubule. Nucleolar hypertrophy.
- FIG. 17. *Black crested cockatoo*. Convoluted tubule. Type A inclusion with margination of basophilic nuclear chromatin.
- FIG. 18. *Wonga Wonga pigeon*. Convoluted tubule. Nuclear hypertrophy.
- FIG. 19. Same. Convoluted tubule. Nucleolar hypertrophy. Atypical crystalline acidophilic inclusion in a large clear area.
- FIG. 20. *Marsh hawk*. Collecting tubule. Large type B inclusion staining also with basic dyes. Nuclear hypertrophy.
- FIG. 21. *Ceropsis goose*. Convoluted tubule. Several types of B inclusions.



LESIONS OF THE CORONARY ARTERIES AND THEIR BRANCHES IN RHEUMATIC FEVER *

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Karsner and Bayless'¹ very comprehensive analysis of the literature dealing with vascular disease occurring during the course of rheumatic fever has made it unnecessary to present a lengthy review of this subject. With the exception of the observations made by Bouillaud^{2, 3} in 1835 and 1840, studies in this field were extremely vague until the latter part of the nineteenth century. This vagueness was due to the fact that the clinical and anatomical concepts concerning rheumatic fever were, for the most part, extremely ill-defined and that the scattered reports on vascular complications were generally based solely on clinical observations.

In spite of Bouillaud's reports, half a century elapsed before Krehl⁴ laid the basis for our modern concept of the relation of rheumatic fever to coronary artery disease. In this amazingly complete report entitled "Beitrag zur Pathologie der Herzklappenfehler" Krehl foreshadowed much of the important recent work on the interstitial, often perivascular, lesions now known as Aschoff bodies, predicted the important relation of these lesions to the clinical conception of heart failure in rheumatic fever, and made the further contribution that in this disease arteriosclerotic changes occur in the larger vessels, with thickenings, infiltrations and intimal proliferations in the walls of smaller vessels.

The name of Romberg⁵ must inevitably be linked with these earlier anatomical investigations on rheumatic fever. In 1894 this observer elaborated on Krehl's descriptions of coronary artery lesions, adding original observations on the occurrence of a diffuse hyaline thrombosis of the smaller arterioles and on the presence of a periarteritis involving a middle sized coronary artery. Since the beginning of the twentieth century many notable contributions have been made in this field, chiefly by Rabé,⁶ Aschoff,⁷ Barié,⁸ Aschoff and Tawara,⁹ Geipel,^{10, 11} Coombs,^{12, 13, 14} Gerhardt,¹⁵ Takayasu,¹⁶

* Aided by a grant from the Lucius N. Littauer Foundation.

Received for publication August 20, 1934.

Douglas,¹⁷ Thalhimer and Rothschild,¹⁸ Fahr,¹⁹ Wätjen,²⁰ Wiesel,²¹ Swift,²² MacCallum,^{23, 24} Pappenheimer and VonGlahn,²⁵ Shaw,²⁶ Talalajew,²⁷ Perry,²⁸ Klinge,²⁹⁻³² and Karsner and Bayless.¹ These workers confirmed the reports of Krehl and Romberg and added a number of new observations on a variety of lesions occurring in the coronary tree in this disease.* With very few exceptions these lesions were not considered specific of rheumatic fever. On the contrary, many of them were held to be similar to those found in other toxic and infectious diseases and in exanthemas (Martin,³³ Landouzy and Siredey,^{34,35} Huguenin,³⁶ Hanot,³⁷ Cowan,³⁸ Wiesel,³⁹ Wiesner⁴⁰).

While the above mentioned contributions cover a variety of lesions which can be observed in the coronary arteries and their branches in the course of rheumatic fever, many of the reports are based on the observation of very few cases, at times on isolated instances. Furthermore, it appears that no systematic attempt has been made to study in detail all the lesions occurring in the active and inactive cases of rheumatic fever on a sufficiently large and representative material from which it might be possible to obtain statistical data on their incidence. The exhaustive study by Karsner and Bayless was undertaken from a different angle. These authors concerned themselves with the incidence of various progressive, retrogressive and inflammatory processes in the coronary tree as a whole, rather than with a description of the individual vascular lesions as they occur in different parts of the coronary system. Furthermore, their studies are confined to the incidence of these phenomena in active rheumatic cases and in normal controls. In this report we propose to classify and describe a number of distinctive vascular lesions found in active as well as inactive cases of rheumatic fever. Consideration will be given to the type of vessel involved and to the incidence of the lesions in various sites in the heart. Since some of these lesions are also found in normal control cases, statistical comparisons will be made with these.

The study is based on an examination of 100 hearts, 66 of which were from cases of acute rheumatic fever with Aschoff bodies in the myocardium and presenting the evidences of activity as outlined by Rothschild, Kugel and Gross⁴¹ in a recent publication. The

* Since submitting this manuscript for publication a report has been published in which several additional vascular lesions of the coronary branches are described.

Fraser, A. D. Coronary endarteritis in acute rheumatism. *Arch. Dis. Childhood*, 1934, 9, 267-276.

remaining 34 hearts were from inactive cases presenting the typical anatomical evidences of inactive rheumatic fever, *viz.* the characteristic valvular deformities with or without evidence of old auricular lesions, but without the presence histologically of swollen collagen, inflammatory cells, Aschoff bodies or fibrinous pericarditis. Fifty carefully studied normal* hearts, representing age periods from birth to the ninth decade, served as a baseline to establish deviations from the normal. The findings in this control series have been published elsewhere (Gross, Epstein and Kugel⁴²). The hearts were generally fixed in 10 per cent neutral formol-saline.† For routine studies the standardized sections of Gross, Antopol and Sacks⁴³ were cut and stained with hematoxylin and eosin and with Weigert's elastic and Van Gieson's connective tissue stains. At times, Mallory's phosphotungstic acid hematoxylin, Weigert's fibrin method and Masson's trichrome stains were employed. The studies on the main coronary arteries were made on blocks removed from the left circumflex coronary artery 1 cm. from its ostium, from the left anterior descending branch 2 cm. from the ostium of the left circumflex coronary artery, and from the right circumflex coronary artery 1 cm. from its ostium.

In this report the vascular lesions of the main coronary arteries and their myocardial branches will be described and statistical data which concern only these vessels will be presented. Brief mention will also be made of the vascular lesions in the valves, and of the lesions in the roots of the pulmonary artery and aorta, together with the vascular lesions found in the pericardial sheaths surrounding these structures, but these will not be included in the statistical data. Studies in this field will be published in separate reports.

The lesions of the coronary artery tree found in active and inactive rheumatic fever can be divided into two categories. (A) *Evolutionary changes also found in normal control cases.* It will be shown that these normally occurring vascular lesions assume considerable significance in rheumatic fever because of their much more rapid and considerably earlier development in this disease. (B) *Lesions occurring either uncommonly, or never, in normal control cases.* It is

* In the selection of this material, diseases that were likely to implicate the coronary vessels were excluded, as were those hearts that showed obviously diseased coronary arteries on gross inspection.

† Solution of formaldehyde U. S. P. 10 parts, 1 per cent sodium chloride solution 90 parts. This solution is rendered neutral with a weak alkali.

not our purpose to lay claim to specificity for any of the lesions falling into this category. In order to do so it would be necessary to study extensively the coronary arteries and their branches in other inflammatory lesions of the heart and in febrile diseases. It is our impression, nevertheless, that many of the vascular diseases falling into this second category are so peculiar and so rarely met with in other diseases that their presence should lead to a strong suspicion of rheumatic fever in its active stages.

This report will be further subdivided into a description of the lesions due to rheumatic fever as they occur in the myocardial arteries* and into those found in the main coronary trunks. For purposes of clarity we shall first present seriatim a description of each lesion with a discussion of the incidence and sites of predilection. This will be followed by a discussion of these lesions considered from the point of view of their occurrence in active and quiescent rheumatic fever.

LESIONS OF THE MYOCARDIAL ARTERIES IN RHEUMATIC FEVER

(A) *Evolutionary Changes also Found in Normal Control Cases*

1. *Intimal Elastification*

By this term is meant a conspicuous increase in the intimal elastic membranes, irrespective of the cause of this increase. Thus, it may be the result of elastic-hyperplastic proliferation; it may follow the development of fibroblastic proliferation; or it may occur concomitantly with the evolution of the musculo-elastic layer. Elastification involving the intima of the myocardial arteries (Fig. 1) has been shown by us⁴² to become fairly prominent during the third decade of life in normal control cases. At this age period it is seen with some regularity in the left posterior papillary muscle where the lesion eventually develops into its most advanced form. It occurs later, less consistently and less markedly, in the posterior wall of the left ventricle and in the interventricular septum. In these sites the lesion becomes prominent during the fifth decade. It occurs with great rarity in the auricular walls and the pulmonary conus. In the inactive rheumatic cases there does not seem to be a conspicuous increase in the incidence of this vessel lesion. In the active

* The coronary artery branches within the myocardium.

cases, however, the lesion occurs at times as early as during the fifth year of life and, in contrast to the normal controls, it may be seen in the pulmonary conus and the auricular myocardium. In the latter, even the arterioles may develop a thickening of the lamella elastica interna and become surrounded by a fairly dense elastic mantle (Fig. 2). This represents, therefore, a remarkable increase in the tempo of development of this normally occurring evolutionary vascular change. Beginning with the latter half of the first decade, intimal elastification can be found in almost every case of active rheumatic infection.

2. *Medial Elastification*

The same remarks apply to the vascular lesion that we have termed "medial elastification" (Fig. 3). In a previous report⁴² we have shown that this lesion, which consists of the development of elastic membranes between the medial cells, does not become prominent until the middle of the third decade, when it is found with great regularity in the left posterior papillary muscle. As with the previously described vascular lesion, it does not appear with any degree of regularity in the posterior wall of the left ventricle and in the interventricular septum until the fifth decade of life. It is rarely found in the auricular myocardium or pulmonary conus. The appearance of this medial elastification in the active cases of rheumatic fever is of considerable interest, not only because of the extremely early age periods during which it may be found in this condition ($4\frac{1}{2}$ years), but also because of its widespread distribution and frequency of occurrence in the left posterior papillary muscle and interventricular septum in these early age periods. It is generally found in the posterior wall of the left ventricle from the beginning of the third decade on. It occurs rather infrequently and later in the auricles and pulmonary conus. As in the previously described lesion, there is no significant difference in the incidence of this lesion as between the inactive cases and the normal control series.

3. *Fibro-Elastification*

This is a term which we have applied to the fusion of the elastic-hyperplastic intima with a heavily elastified media. Simultaneously, there generally takes place a disappearance of smooth muscle with replacement by connective tissue. The resulting picture is one of

complete metamorphosis of the vessel in which the predominating features are fibrosis and elastification with or without the presence of irregular islands of smooth muscle tissue. The latter in turn may represent remains of the media or of the smooth muscle intimal layer (musculo-elastic layer). In its more marked form the topography of the vessel is markedly distorted (Fig. 4), the elastic membranes are irregularly thickened, frayed and ruptured, and present a very bizarre appearance. From the beginning of the third decade on, this type of lesion is found with great frequency in normal controls in the left posterior papillary muscle, and somewhat less regularly in the interventricular septum from the beginning of the fifth decade on. It occurs in exceptional instances in the posterior wall of the left ventricle in the later decades and is rare or absent in the other cardiac sites. It is found somewhat earlier but not frequently in inactive rheumatic cases in the left posterior papillary muscle, interventricular septum and the posterior wall of the left ventricle. It has been observed involving vessels of the interventricular septum at the age of 15 and has also been seen rather infrequently in the pulmonary conus. It has not been observed in the auricular myocardium in our inactive rheumatic material. In sharp contrast to this, however, is the appearance of this lesion in the active rheumatic cases where it has been seen as early as the age of $4\frac{1}{2}$ years in the left posterior papillary muscle. It appears with greater regularity at about the age of 15 years. From the age of 20 years on it is seen quite prominently and almost invariably in the left posterior papillary muscle and in the interventricular septum. The lesion has also been seen in these active cases, though infrequently, in the pulmonary conus. It has not been observed in the auricular myocardium. It is the appearance of this very conspicuous, normally occurring lesion several decades earlier than is to be expected, which best exemplifies the influence of rheumatic fever in hastening normal evolutionary vascular changes.

4. Adventitial Fibrosis

This lesion, which Wild ⁴⁴ has recently emphasized in connection with rheumatic fever, consists of the development of a dense, wide, adventitial sheath. The vessel wall is seen surrounded by a conspicuous circular or oval zone of fibro-elastic tissue which may merge with neighboring scar tissue. Adventitial fibrosis is seen not in-

frequently in the active cases and very frequently in the inactive cases. It is generally difficult to distinguish it from unusually wide adventitial layers observed in control cases. However, in rheumatic fever, particularly in the inactive stages, it is found in considerably earlier age periods where it may be quite extensive. The sites of predilection are the left posterior papillary muscle and the interventricular septum. It occurs with lesser frequency in the posterior wall of the left ventricle.

(B) Lesions Occurring either Uncommonly or Never in Normal Control Cases

5. Medial Glassy Hypertrophy

In approximately half of the active cases of rheumatic fever, one encounters in the smaller myocardial arteries and in the arterioles, practically throughout the heart, a swelling of the smooth muscle cells which take on a glassy appearance and present rather indistinct outlines of the cell walls (Fig. 5). The nuclei tend to occur in clusters and become somewhat rounded. The nuclear membrane and chromatin particles are quite sharp and distinct. This lesion is extremely uncommon in the inactive cases. It is rare in the control cases but has been noted repeatedly in other inflammatory diseases in the myocardium and in febrile conditions. It is obviously non-specific and is an evidence of an irritative process. There does not appear to be any special site of predilection for this lesion.

6. Medial Hypertrophy

This is an extremely uncommon lesion in normal controls where a hypertension, past or present, can safely be ruled out. It is represented by very obvious thickening of the vessel wall, due to an increase in the number and size of the smooth muscle cells. The lesion occurs very rarely in the inactive cases. It is one of the most frequent findings in the active cases and is seen particularly prominently in the retro-aortic myo-epicardial auricular wedge.* It occurs less frequently in the posterior wall of the left ventricle and in the interventricular septum. Hypertrophied vessels of this type are also frequently found near the septum fibrosum, in the pericardial tissue

* A. M. V. section of the standardized blocks. ⁴³

close to the auriculoventricular groove, in thickened rheumatic valves and in the subaortic angle. Of interest is the fact that in the active cases this vascular lesion occurs with almost the same frequency in young (17 months) and old.

7. *Intimal Fibrosis*

One of the most frequently occurring coronary vascular lesions in rheumatic fever, confined largely to the active cases, is a thickening of the intima due to fibroblastic proliferation (Fig. 6). While intimal fibro-elastic changes are frequently encountered in the normal control series, particularly in the later age periods, the development of a clean-cut concentric or eccentric widening of the intimal layer by a zone of non-elastified connective tissue has not been found in these controls. In active rheumatic infection this lesion is distributed, though somewhat irregularly, throughout the myocardium. Its site of predilection is the left posterior papillary muscle, where it is found quite regularly, even in the earliest age periods. The lesion apparently occurs quite rapidly in active rheumatic fever. As a consequence, it may be observed in those vessels that have not as yet developed elastic-hyperplastic changes. Undoubtedly this fibroblastic layer eventually becomes elastified. Indeed, it seems reasonable to assume that intimal fibrosis is not infrequently the precursor of intimal elastification in cases of rheumatic infection. Since it occurs even in the youngest cases it may account for the early development of intimal elastic changes in these cases. The fibrosis is not infrequently eccentric and may lead to marked narrowing of the vessel. Superimposed on this fibrotic intima one may at times observe a swelling and proliferation of the endothelial cells.

Intimal fibrosis may be superimposed on intimal elastification. In such cases there develops internally to the innermost intimal elastic lamella a distinct zone of varying width consisting of collagen and connective tissue cells (Fig. 7). The layer is in turn lined by endothelium. This rather sharp lamination of the intima by elastic and fibrotic zones suggests that they are the results of older and more recent rheumatic bouts.

8. *Giant Medial Hypertrophy with Metallaxis*

This lesion is represented by a conspicuously and unusually florid hypertrophy of the media (Fig. 8). The cells are generally somewhat irregularly arranged (metallaxis) and may or may not present discontinuous elastic membranes between them when these are present. The chief differences between what we have termed "giant medial hypertrophy with metallaxis" and the lesion described under the term "medial hypertrophy" lie in the considerably greater extent of the hypertrophy, the irregularity in the planes of the muscle fibers, the frequently irregular distribution of the elastic membranes when present, the occasionally vague and bizarre appearance of the inner aspect of the adventitial layers, and frequently in the presence of edematous spaces between the medial smooth muscle cells. A lesion somewhat resembling giant medial hypertrophy was seen in the left posterior papillary muscle and in the interventricular septum in only 2 out of the 50 normal control cases at the ages of 23 and 45 years respectively. In the inactive rheumatic cases it was not found. On the other hand, the lesion was observed in 8 of the 66 active cases, generally in the retro-aortic myo-epicardial auricular wedge, where it has been found as early as at the age of $3\frac{1}{2}$ years. It is found with greater frequency in the pericardial sheath surrounding the root of the pulmonary artery but, as has been indicated, in this report the vessels in this site will be considered separately from the myocardial arteries proper. Because of the infrequency of this vessel lesion in the control group, it seems best to include it in the second large group of our classification.

9. *Medial Edema*

Besides the "giant medial hypertrophy with metallaxis," several other vascular lesions, especially the arteritides, and particularly those associated with necrotization (*vide infra*), showed a conspicuous edema of the media (Figs. 11, 16, 23, 24, 25, 26, 28). If one bears in mind the artifacts created by tissue fixation, genuine edema of the media is relatively infrequently encountered. It occurs somewhat more often in the main coronary arteries. This will be discussed further. Incidentally, a cross-section of the intimal musculo-elastic hyperplastic lesion gives the impression of the "état reticulaire" of the media quoted by Karsner and Bayless. Longitudinal sections

of such vessels stained for elastic tissue disclose that the reticular appearance is due to intimal longitudinal smooth muscle cells which may be hypertrophied, somewhat swollen and pale staining.

10. *Arteritis (Exudative and Necrotizing)*

Under the term "arteritis" there is considered definite inflammatory infiltration of the vessel wall with or without necrosis, and without the distinctive histological features that characterize the special forms to be described. The lesions vary from a relatively mild infiltration of one of the main layers of the artery with lymphocytes and the presence of edema, to an active destructive infiltration with polymorphonuclear leukocytes (Fig. 9), plasma cells and, at times, basophilic cells characteristic of the Aschoff body. As noted by VonGlahn and Pappenheimer,⁴⁵ many of the wandering cells are oriented in the direction of the lumen. Fibrinoid swelling of the collagen may be present. The lesion may be so extensive and destructive and the media may undergo such conspicuous necrosis that a picture is produced which is indistinguishable from the necrotizing arteritis of so-called polyarteritis nodosa. Together with these inflammatory and destructive phenomena there may be seen irregular proliferations of the intimal endothelium or of the mesenchymal tissue lying beneath the endothelium. These cells may be arranged in palisade form and the internal and external elastic membranes may assume a glassy swollen appearance or become ruptured and disappear. Thrombi have been observed within the lumens of such actively inflamed vessels.

There does not appear to be any advantage in subdividing the various grades of exudative and necrotizing arteritis occurring in rheumatic fever. While arteritis of this type was never found in the inactive cases, it was observed in 11 of the 66 active cases examined, where it occurred chiefly in the interventricular septum with about an equal distribution in the other cardiac sites. In 4 of these cases (reported in detail by Friedberg and Gross⁴⁶), the lesions were so extensive and associated with such definite involvement of the vessels elsewhere in the body that the cases were considered polyarteritis nodosa associated with rheumatic fever.

Apart from these 11 cases presenting various forms of exudative and necrotizing arteritis in the myocardial arteries, 8 additional

cases displayed the special forms of arteritis to be described (*vide infra*). Of this total of 19 cases, 8 also presented inflammatory phenomena in the main coronary arteries. In 2 additional cases the inflammatory lesions were confined to the main coronary arteries. In 1 additional case a fibrinoid necrosis of the coronary vein was noted, and in 1 other a verrucous endophlebitis associated with inflammatory lesions of the myocardial arteries. These figures on the incidence of the lesions in the veins are undoubtedly too low, since no systematic study was made of the venous system. Disregarding the latter, therefore, it would appear that 33 per cent (22 out of 66 cases) of the active cases presented some form of arteritis in the arterial tree of the heart. It should be noted that this figure is also exclusive of the inflammatory lesions in the pulmonary artery and aorta.

11. Subendothelial Hemorrhagic Arteritis

This corresponds to one of the vascular lesions described by Von Glahn and Pappenheimer⁴⁵ as occurring in a variety of tissues including the aortic valve. It consists essentially of a hemorrhage beneath an otherwise intact, sometimes swollen endothelium, raising it away from the vessel wall (Fig. 10). The lumen may contain a hyaline thrombus and the wall show moderate infiltration with inflammatory cells. This lesion was not encountered in the normal controls or in the inactive cases. It was found in 4 active cases (in 2 it occurred in the retro-aortic myo-epicardial auricular wedge) and may possibly represent a specific lesion of rheumatic fever.

12. Net-Like Fibrinous Thrombosis Affecting Small Myocardial Arteries

This is another vascular lesion described by the above mentioned authors as occurring in the small peripheral arterioles and capillaries, and considered by them specific of rheumatic fever. As observed by them in a variety of organs, it is "characterized by the exudation of fibrin into and about the vessel, by destructive changes in the cellular components of the vessel wall, by a distinctive cellular reaction in the adjacent tissue and by the absence of thrombosis. These acute lesions are followed by organization with or without formation of new collateral channels within the thickened intima and occasionally within the muscular layer." A somewhat similar

lesion was noted by them about a small capillary in the heart. The vessel over a small area was surrounded by a mass of fibrin which infiltrated the surrounding tissue. At the margin of the fibrin there were many Aschoff cells and a few polymorphonuclear leukocytes. The lumen was not occluded by thrombus and the endothelium was intact.

We observed this lesion in the retro-aortic myo-epicardial wedge of the right auricle in 1 case. Here, the inflammatory phenomena in the wall of the vessel and within the periadventitial tissue were limited in their extent. The most characteristic feature was a net-like formation of fibrin within the lumen of the vessel (Fig. 11), thus differing from the typical blood platelet thrombus. As noted by VonGlahn and Pappenheimer, in the organization of this lesion the fibrin network is apparently displaced by connective tissue with the formation of a cavernous type of canalization.

13. *Endarteritis Verrucosa*

In 1924 and 1926, Holsti^{47, 48} described peculiar proliferative lesions occurring as finger-like processes within the vessel lumens of tonsils and the gastro-intestinal tract in "arthro-nephro-cardiopathies." Under the title "arteriitis verrucosa," Holsti⁴⁹ described the mechanism of their formation as being either primary, representing a vascular lesion *sui generis*, or secondary to such vascular changes as lead to a thrombosis. The term "endarteritis verrucosa," as employed by us, refers to the formation of typical endarterial verrucae probably identical with those seen on active rheumatic valves (Fig. 12). While the material available for study of this lesion was too limited to permit an exhaustive investigation of the mechanism of its formation, it would appear that a fibrinoid necrosis of the intimal layers of the vessel with fusion of the various histological elements may be followed by, or associated with, deposition of blood platelet thrombi. This eventually leads to organization. If the original verrucous lesion is large and polypoid, the organized plug is eventually represented by a finger-like connective tissue process within the lumen of the vessel — a lesion which we shall refer to as "endarteritis polyposa." The organized verrucous plug may be attached to the vessel wall at several points, producing a canalization of the lumen. Thus, it will be seen that this lesion oc-

cupies an intermediate position between necrotizing arteritis on the one hand and intravascular thrombosis on the other. Typical endarteritis verrucosa was found in the myocardial arteries only in 2 active cases, where the individuals were $4\frac{1}{2}$ and 10 years old respectively. There was no special site of predilection. In addition, it was seen in the left anterior descending coronary branch in 1 case.

14. *Granular Plugged Vessels*

This lesion bears a superficial resemblance to the early stages of endarteritis verrucosa and polyposa. In its typical form it consists of a spherical, oval or lobated, finely granular mass which partially or completely occludes the lumen of the vessel (Fig. 13). It is generally covered by a somewhat swollen endothelium and frequently contains several nuclear ghosts within its substance. It would appear that the lesion is produced by proliferation of vascular endothelium with swelling and granular transformation of the protoplasm, fusion of the cells and degeneration of the nuclei, a process described as occurring in atypical verrucous endocarditis (Gross⁵⁰). In the healing of these granular masses organization takes place with replacement by connective tissue. The end result may therefore be a parietally situated fibrous plug within the lumen of the vessel, a centrally situated polypoid mass, in which stage it is classified as endarteritis polyposa, or a canalization of the lumen. Granular plugging of the myocardial arteries was observed in only 3 active cases, where the individuals were 15, 25 and 38 years respectively. The site of predilection appeared to be the interventricular septum. One of the cases (age 25 years) showed, in addition, an extensive and almost completely occluding granular plugging of the left circumflex coronary artery (Fig. 28).

15. *Thrombosis*

Thrombosis of myocardial arteries directly attributable to rheumatic injury of the vessel rather than to a primary or secondary sclerotic vascular damage was seen in only 4 active cases. Three of these (age $7\frac{1}{2}$, 10 and 30 years) were associated with a marked necrotizing arteritis (polyarteritis nodosa). In one of these (age $7\frac{1}{2}$ years), there was also present a thrombosis of the left circumflex coronary artery. In the fourth case (age 17 months), also asso-

ciated with an arteritis (but not of the necrotizing type), large branches of the left circumflex coronary artery showed the presence of occluding typical blood platelet thrombi and there was present extensive myocardial infarction. This case also presented a thrombosis of the main left circumflex coronary trunk (Fig. 27). While on this subject, it is pertinent to mention that red blood cells and sometimes fibrin plugs are not infrequently encountered occupying the lumens of various small coronary vessels in active rheumatic fever. While it is at times tempting to call these thrombi, we have reserved this designation for the typical layered blood platelet intravascular collections which definitely are of *intra vitam* formation and which are partially or completely occluding and generally associated with myocardial infarction of various degrees. As in the case of endarteritis verrucosa, organization of typical thrombi results either in parietally or centrally situated, partially or completely occluding connective tissue plugs, or, polypoid masses may be formed within the lumen of the vessel, or finally, canalization may take place.

16. Canalized Vessels

It was already mentioned that the healed lesions following net-like thrombosis, endarteritis verrucosa, granular plugging of vessels and thrombosis may be represented by a canalization of the vessel lumen (Figs. 13, 14). As a consequence, these lesions have been found only in the active cases and occur somewhat less frequently than the corresponding lesions which produce them. These canalized vascular lesions have also been observed in atypical verrucous endocarditis (Gross⁵⁰).

17. Endarteritis Polyposa

As has already been indicated, this term is applied to the end stages of a variety of lesions. It consists of a polypoid or finger-like plug of organized or organizing connective tissue which is situated within the lumen of a vessel and is separated from the vessel wall throughout its greater extent (Fig. 15). It may result from endarteritis verrucosa, granular plugging or genuine thrombosis. It was observed only in 3 active cases where the individuals were 17 months, 10 years and 38 years respectively.

18. *Aschoff Bodies Associated with Blood Vessels*

The frequent, but by no means invariable, proximity of Aschoff bodies to blood vessels has been repeatedly emphasized. These may assume a very intimate relation to the vessel wall. In rare instances other observers have noted an apparent compression of the vessel lumen by this lesion. Karsner and Bayless believe that this compression has little if any influence on the circulation within. Rather infrequently, irregular basophilic cells, somewhat resembling those of the Aschoff body, are found within the media. It is very likely that in many cases these represent merely hypertrophied and somewhat altered medial smooth muscle cells. As already mentioned, eventual transformation of the Aschoff body into connective tissue scar undoubtedly accounts for most of the adventitial scarring.

19. *Intimal Musculo-Elastic Hyperplastic Lesion*

While the development of smooth muscle cells within the elastic-hyperplastic layers of the intima is a normal evolutionary process, the cells are relatively few in number, seldom attain more than one or two layers in thickness and are generally somewhat irregularly distributed. Moreover, the elastic-hyperplastic changes are not infrequently more conspicuous than the smooth muscle elements. In distinct contrast to this is the "intimal musculo-elastic hyperplastic lesion." This is one of the most characteristic vessel lesions seen in rheumatic fever and consists of the development of numerous layers of longitudinal smooth muscle cells which lie internally to the lamella elastica interna and are covered by a generally swollen, sometimes raised endothelium (Fig. 16). These smooth muscle cells frequently show mantles of elastic tissue which, on cross-section, present a honeycombed appearance. This intimal musculo-elastic zone is generally concentrically placed, may be eccentric (Fig. 17) and is often so extensive as to occlude almost completely the vessel wall. In some instances, the smooth muscle cells are very large and pale staining.

As with other intimal vascular changes in rheumatic infection, the end state of the intimal musculo-elastic hyperplastic lesion appears to be an elastification (Fig. 18). This consists of a conspicuous increase of elastic tissue which, together with connective tissue, even-

tually replaces the smooth muscle elements. At times, the intima contains several, irregular, laminated layers of musculo-elastic tissue separated by heavier elastic membranes (Fig. 19). Such lesions suggest the possibility that these layers represent repeated attacks of rheumatic fever.

The development of a somewhat similar musculo-elastic hyperplastic layer, but in considerably abbreviated form with the smooth muscle cells rather irregularly arranged, has been seen in one of our normal hearts at the age of 47, and in one at the age of 65 years. Both occurred in the posterior wall of the left ventricle. In one other case, aged 9 months, a vessel of this type was seen in the pericardial sheath surrounding the pulmonary artery. In the inactive rheumatic cases the lesion was seen once in the interventricular septum in a patient aged 26, once in the retro-aortic myo-epicardial auricular wedge in a patient aged 68, and once in the aortic pericardial mantle in a patient aged 30 years. In the active rheumatic group it was seen in approximately half of the cases. Its distribution was chiefly in the retro-aortic myo-epicardial auricular wedge and in the region of the septum fibrosum, including the posterior portion of the interauricular septum. The next most frequently involved site was the pericardial sheath surrounding the pulmonary artery. The lesion was least frequently seen in the posterior wall of the left ventricle, in the left posterior papillary muscle, pulmonary conus and interventricular septum. Apart from these myocardial sites this lesion is found in the subaortic angle and in thickened rheumatic valves, where it occurs with considerable frequency.

The occurrence of these intimal musculo-elastic lesions in greater numbers and in exaggerated form is so highly characteristic that, with the possible exception of syphilis, in which a somewhat similar lesion has occasionally been observed, their presence is highly suggestive of active rheumatic involvement. This is particularly true when the intimal musculo-elastic hyperplastic lesions, together with others which have been described above, are found in the left auricular myo-epicardial wedge (Fig. 20) and in the region of the septum fibrosum, including the conduction system.

LESIONS OF THE PULMONARY ARTERY AND AORTA

Besides the above mentioned findings, various inflammatory lesions, vascularization and scarring, occur with remarkable regu-

larity in the pulmonary artery and aorta in both the active and inactive cases. This will be taken up in a separate report. It is noteworthy, however, that in the pericardial sheath behind these vessels one very frequently encounters medial hypertrophy, giant medial hypertrophy with metallaxis and intimal musculo-elastic hyperplastic lesions. These are seen with conspicuous frequency in the active cases and form a characteristic part of the picture presented by blood vessels in rheumatic fever.

In presenting the above mentioned findings we have deliberately avoided including in our statistical data the vessel lesions found within the valves, subaortic angle and subendocardium. These are generally of the medial hypertrophied, giant medial hypertrophied, intimal musculo-elastic hyperplastic and thickened granulation tissue capillary types. By themselves, they form conspicuous vascular lesions, but inasmuch as they represent, in our opinion, the end stages of newly formed vessels, we have not considered it justifiable to include them in the group of vascular lesions affecting the pre-existing coronary arteries and their branches.

LESIONS IN THE MAIN CORONARY ARTERIES DUE TO RHEUMATIC FEVER

Inactive Cases

We have already shown ⁴² that in normal control cases all three layers of the coronary arteries undergo a series of evolutionary changes which present considerable variations, not only during the various age periods but also in individual cases. It appears that the rate of development of these changes may vary markedly from individual to individual. It is, as a consequence, extremely difficult to determine whether the main coronary arteries display these changes in a given case in a more marked form and earlier, because of the rheumatic involvement. It appears, nevertheless, that even in inactive rheumatic cases there is a tendency toward an earlier development of heavy intimal elastic-hyperplastic and fibrotic layers, and for the appearance of heavier elastification and scarring of the media.

The elastic tissue in the media of normal coronary arteries appears to increase during the early age periods and decrease thereafter. Furthermore, while there is a certain amount of cross-weaving

of the elastic fibers, they run as a whole parallel to the main axis of the lumen. In the inactive rheumatic cases the elastic tissue shows a consistent increase in amount from the early age periods on, and the direction of the fibers not infrequently shows considerable distortion and irregularity, appearing as scattered, heavily elastified patches (Fig. 21). This condition is not to be confused with the patchy medial elastification appearing under sclerotic intimal plaques. Nor is it to be confused with the irregular reticular elastified areas found not infrequently in the media of normal coronary arteries and associated with the presence in the media of what we have termed "intermediary layers."⁴² One of the illustrations in the article by Karsner and Bayless appears to represent such a normally occurring vascular architectural variant.

Discontinuities in the medial elastic membranes are not infrequent, particularly after the third decade of life. Before concluding that there is present rupture of these membranes in a given case, the normally occurring discontinuities should be borne in mind, and only when there is present a very obvious disruption of these membranes in areas which do not contain the "intermediary layers," should this diagnosis be made. Rupture of the medial elastic membranes is relatively infrequent in the inactive cases. When it occurs it is generally associated with scarring.

Similar remarks apply to scarring of the media. This can be considered as of rheumatic origin only in obviously scarred areas (larger and more irregular than the normally occurring fibrotic mosaic), when they are focal in their distribution and not situated directly beneath an arteriosclerotic plaque with lipoid and calcific deposition (Fig. 22). Considered along these lines, moderate scarring and elastification of the media occur either in the left circumflex coronary artery, right circumflex coronary artery, or the left anterior descending coronary artery in approximately one-third of the inactive cases. It was marked in four of these. There did not seem to be a predilection for any one of these three main vessels.

We have been discouraged in our attempts to evaluate the changes in the amount of chromotropic substance to be found in the coronary arteries. It appeared to us that the technical factors involved, and the uncertainty in estimating these changes, rendered such a study too much open to criticism. It should be mentioned, however, that whereas Karsner and Bayless were unable to demonstrate this sub-

stance in the coronary vessels of control cases, they observed it frequently in their active rheumatic material. With regard to the presence of medial edema it may be said that its occurrence in sites not in the immediate vicinity of sclerotic intimal plaques was rather infrequent and too ill-defined to permit of reasonably accurate appraisal.

It is as well to mention here that we have been rather strict in our definition of "fibrinoid change." Karsner and Bayless define this as "an intensely acidophilic substance, arranged in fibrillar fashion, sometimes with beading at intersections." According to this definition these authors observed fibrinoid change in many of their control cases. However, unless the tissue showed distinct swelling with loss of outlines and unless the tinctorial alterations were very marked, we have not used the term "fibrinoid change." With this somewhat stricter definition in mind, we have not been able to demonstrate these changes either in the normal control cases or in the inactive cases.

Active Cases

While the above mentioned changes are the only ones that we have been able to observe in the inactive cases, and we repeat that one can offer little more than a suggestion of this increased tempo of vascular retrogression, these phenomena seem to occur more frequently and to appear much earlier in the active cases. Noteworthy differences from the former are the greater incidence and extent of edema in the media, even in the absence of an arteritis (Fig. 23); the relative paucity of elastic fibers in the thickened intima, particularly in the earlier age periods, and the presence in a fair number of cases of what we have termed "reduplicated intimal layers." This consists in the early stages of a generally loose, somewhat myxomatous tissue, covered by endothelium and situated on the innermost aspect of the intima (Fig. 24). This layer is seen to be distinctly superimposed on the intima proper, and in later stages apparently becomes elastified. When present, this reduplicated intimal layer may possibly represent one of the results of an attack of rheumatic fever.

The most obvious and distinctive lesions of the main coronary arteries which are found only in the active cases are the exudative, necrotizing and other peculiar types of arteritis similar to those de-

scribed as occurring in the myocardial arteries. These lesions occurred in 10 of the 66 cases (15 per cent). The exudative lesions consist of inflammatory involvement of varying grades of intensity, generally affecting the media. In some forms there may be seen marked edema of the media (Fig. 24) with swelling of the smooth muscle fibers, rounding of the nuclei with prominence of the chromatin, and the presence of inflammatory cells. In other forms of exudative arteritis the predominating cell type is the polymorphonuclear leukocyte, sometimes the lymphocyte. These cells occur either diffusely, sometimes more abundantly seen toward the intimal aspect of the vessel, or are localized in one arc of the circumference of the vessel. Ameboid streamers of polymorphonuclear leukocytes and monocytes are almost invariably seen. The latter sometimes contain fairly large nuclei with rather abundant basophilic cytoplasm. Eosinophiles and mast cells are seen less frequently and irregular basophilic cells, somewhat resembling those found in Aschoff bodies, are rare.

Together with the inflammatory cell accumulations, there was sometimes found a fibrinoid swelling of the collagen. Rupture and dissolution of the elastic membranes occurred only in the very active cases (Fig. 25), particularly in those associated with polyarteritis nodosa (Fig. 26). Pictures simulating rupture of the elastic membranes were occasionally found. Their significance has already been discussed under the findings of the main coronary arteries in the inactive cases.

Both in the active and inactive cases, inflammatory cells, generally lymphocytes, were not infrequently met with in the adventitial and periadventitial layers. In the active cases this was a more frequent occurrence, where also polymorphonuclear leukocytes, monocytes and, at times, Aschoff bodies were also present. It is of interest that these adventitial and periadventitial infiltrations occur even in the absence of macroscopic pericarditis. However, even in the inactive cases, microscopic pericarditis, *i.e.* diffuse, rather mild lymphocytic infiltrations of the visceral pericardium, occurs with great frequency.

While all three main coronary trunks, *viz.* the right circumflex, left circumflex and left anterior descending, were seen involved in some of these cases, it appears that of the three the lesions were found with about the same frequency in the left circumflex and

left anterior descending trunks, and least often in the right circumflex coronary artery. Apart from the above mentioned forms of exudative and necrotizing arteritis (seen in 6 cases), proliferation of the intimal endothelial and subendothelial cells with formation of palisades was found in the left circumflex coronary artery in 1 case (age 6 years), occluding thrombosis of the left circumflex coronary artery (Fig. 27) with myocardial infarction in 2 cases (age 17½ months and 7½ years), verrucous endarteritis involving the left anterior descending branch in 1 case (age 9 years), and a granular plugged lesion (Fig. 28) almost completely obliterating the left circumflex coronary artery in 1 case (age 25 years). Of great importance is the fact that, with the exception of the last mentioned lesion, all the inflammatory phenomena found in the main coronary vessels in active rheumatic fever occurred during the first 15 years of life. This affords additional evidence of the extraordinarily dramatic course taken by this disease in the young, a point of perhaps some immunological significance.

Returning to a consideration of the changes in the main coronary arteries in active cases, which are similar to those found in inactive, as well as in normal control cases, one notes, as mentioned before, the development of increased medial elastification (as early as 6½ years) and a much earlier and considerably advanced tempo in the formation of intimal hyperplasia. An illustration of the latter is a case where the individual was 15 years of age, which showed an intimal thickening approximately four times the size of the media in the right circumflex coronary artery. This process normally appears during the fourth decade of life. In another case (age 29 years) the left circumflex coronary artery showed an intima ten times the thickness of the media, whereas it is generally twice the thickness of the media at this age period. A not inconsiderable number of cases similar to these mentioned, emphasizes the fact that besides active inflammatory and destructive phenomena, rheumatic fever induces in the walls of the main coronary arteries a series of precocious evolutionary metamorphoses which cannot be differentiated from those occurring normally as the result of age.

DISCUSSION

Consideration of the lesions described in this report makes it evident that both from the point of view of their frequency and of their

extent, the coronary arteries and their branches in the heart show very marked damage due to rheumatic fever. This damage is very vivid and impressive in the active cases. Indeed, it may be so extensive that it would be difficult if not hazardous to venture an opinion as to whether the vascular or primary myocardial injury is the more significant with regard to the life of the patient.

In the inactive cases the lesions do not appear to be essentially dissimilar from the normally occurring evolutionary age period changes. As has been indicated, however, a significant difference lies in their somewhat more rapid development. Since these changes, both in the myocardial arteries as well as in the main coronary branches, merge imperceptibly with what is considered arteriosclerosis, it would appear that in the majority of instances the entire vascular damage and its consequences in these inactive cases may be attributed to an earlier fibro-elastification of the myocardial arteries in rather important sites of the heart (where ischemia is more likely to occur) as well as to the earlier appearance of arteriosclerosis in the main coronary trunks. This point of view has been recently stressed by Zeek⁵¹ and by Karsner and Bayless.¹

It has already been mentioned in a previous publication⁴² that fibro-elastification of vessels, particularly in the interventricular septum and posterior wall of the left ventricle, may be of significance, inasmuch as these vessels become transformed into more or less passive rigid tubes, little if any subject to vasomotor control. If this process should take place so early in life and so rapidly that there has been very little opportunity for the development of compensatory anastomotic channels, the consequences are likely to be those following myocardial ischemia. How great a rôle, if any, this mechanism, apart from lesions of the main trunks, may play in accounting for some of the symptoms of angina pectoris occurring in a number of these inactive rheumatic cases, is a matter for conjecture.

The findings in the main coronary arteries in the inactive cases are, however, by no means as strikingly clear-cut as are those in the active cases. Here, besides the considerably earlier development of intimal hyperplasia (often of a fibrotic type) and medial elastification and scarring, there also occurred in a significant incidence (15 per cent) a variety of arteritides which are represented by various grades of exudative and necrotizing inflammation, by thrombosis,

as well as by certain peculiar vascular and intravascular lesions, *viz.* edema, palisade formation, endarteritis verrucosa and granular thrombotic lesions. Together with these lesions in the main coronary trunks, the smaller vessels distributed throughout the myocardium presented similar changes in 19 cases. The total incidence of the various types of arteritis occurring in either the main coronary trunks, the smaller myocardial vessels or both, was 33 per cent. The incidence of these lesions in various cardiac sites in order of their frequency was as follows: the interventricular septum, the retro-aortic myo-epicardial wedge, particularly in the region of the septum fibrosum, the posterior wall of the left ventricle, the left posterior papillary muscle and the pulmonary conus.

Furthermore, in these active cases the myocardial vessels displayed a number of normally occurring evolutionary changes more frequently, more extensively and considerably earlier than those found in normal control cases. There was also present a series of degenerative, productive and proliferative changes which are either uncommonly or never seen in normal cases. The clinical implications of such widespread vascular damage are obvious.

There remains to be discussed the possible relation of the lesions occurring in active cases to those found in the inactive cases. An examination of the sites of predilection of the lesions occurring in the active cases discloses that some of the non-arteritic lesions closely parallel the incidence of the lesions found in the inactive cases. This suggests a sequential relationship. The observation that other non-arteritic lesions occur almost exclusively in the active cases and the fact that these were on the whole from considerably younger age periods than the group comprising the inactive cases, suggests an eventual transformation of these non-arteritic lesions into the types seen in the inactive cases. This is probably brought about by a process of elastification. The possible relationship which these events may bear on an earlier development of an arteriosclerotic process has already been discussed. On the other hand, the arteritic lesions show little if any parallelism in their sites of predilection with the lesions found in the inactive cases. One must conclude either that they may heal with little discernible residua or the patients affected with such destructive lesions are less likely to reach the protracted inactive stages of the disease.

From the anatomical point of view the realization that there occur

in the smaller branches of the coronary arteries many different types of vascular lesions, only four of which are similar to those found as normal evolutionary changes, that a number of these are active and destructive, and that some are of such unusual appearance that they possibly represent specific entities, brings to the pathologist additional evidence on which to build up the curiously complex picture which constitutes the anatomical lesions of rheumatic fever. A consideration of the variety and wide distribution of these lesions affords additional support to the belief that the heart is the most extensively involved organ in the body in this disease. It appears further that the various rheumatic vascular affections of the heart produce their damage during the active as well as the inactive stages. During the active states the acute inflammatory and destructive lesions occur. In the quiescent periods the heart is handicapped by the considerably advanced age period changes which occur in the coronary tree.

SUMMARY AND CONCLUSIONS

A description has been presented of the vascular lesions in the main coronary arteries and their branches as they occur in active and inactive rheumatic fever, together with a statistical indication of the frequency of their occurrence, sites of predilection and comparison with the findings in normal control cases. The lesions in the myocardial branches have been classified under a number of different headings. Four of these are similar to the evolutionary age period changes found in normal control cases. Several of the remaining lesions are so peculiar in their structure as to suggest the possibility that they may be specific of rheumatic fever. This conclusion, however, cannot be accepted as final without an extensive search for similar lesions in other affections of the myocardium.

A discussion is given of the mechanism concerned in the development of these lesions, of their possible relation to the development of the arteriosclerotic process and of their clinical significance.

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DESCRIPTION OF PLATES

PLATE 32

FIG. 1. Myocardial artery in posterior papillary muscle showing intimal elastification. Age 38 years. Active case. Medium power. Weigert's elastic and Van Gieson's connective tissue stain.

A = elastified intima; B = media; C = lumen of vessel containing red blood cells; D = myocardium.

FIG. 2. Myocardial arterioles in left auricle showing thickening of lamella elastica interna and formation of external elastic limiting membrane. Age 13 years. Active case. High power. Weigert's elastic and Van Gieson's connective tissue stain.

A = typical arteriole showing heavy internal and external limiting elastic membranes.

FIG. 3. Myocardial artery in posterior papillary muscle showing medial elastification. Age 38 years. Active case. High power. Weigert's elastic and Van Gieson's connective tissue stain.

A = enormous development of elastic fibers between smooth muscle cells of media; B = lamella elastica interna.

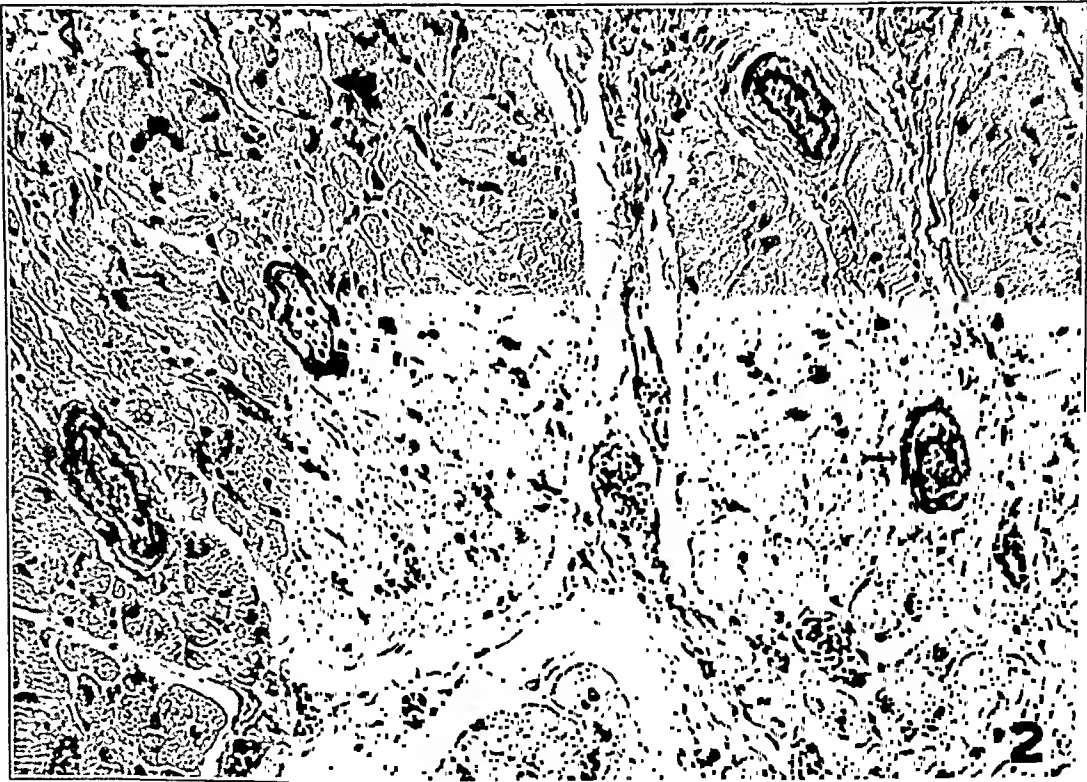
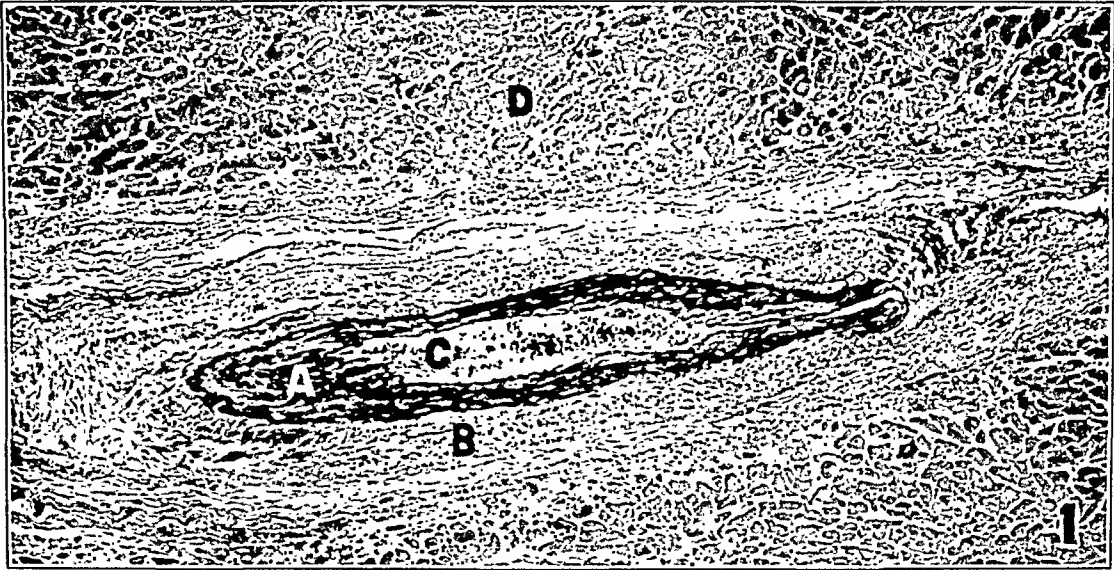


PLATE 33

FIG. 4. Myocardial artery in posterior papillary muscle showing fibro-elastification. Age 38 years. Active case. High power. Weigert's elastic and Van Gieson's connective tissue stain.

A = marked distortion, fraying and rupture of elastic membranes of fused media and intima; B = disoriented, medial smooth muscle cells; C = irregular scarred area of media; D = fused elastified intima and media with complete disappearance of smooth muscle cells; E = adventitia; F = myocardium.

FIG. 5. Small myocardial artery in posterior papillary muscle showing glassy hypertrophy of media. Age 14 years. Active case. High power. Weigert's elastic and Van Gieson's connective tissue stain.

A = lumen of vessel containing red blood cells. Note swollen endothelial cells. B = fused medial smooth muscle cells; C = rounding and clumping of nuclei; D = myocardium.

FIG. 6. Myocardial artery in posterior papillary muscle showing intimal fibrosis. Age 15 years. Inactive case. Medium power. Weigert's elastic and Van Gieson's connective tissue stain.

A = fibrotic intima; B = hypertrophied media; C = intimal elastification in another vessel; D = myocardium.

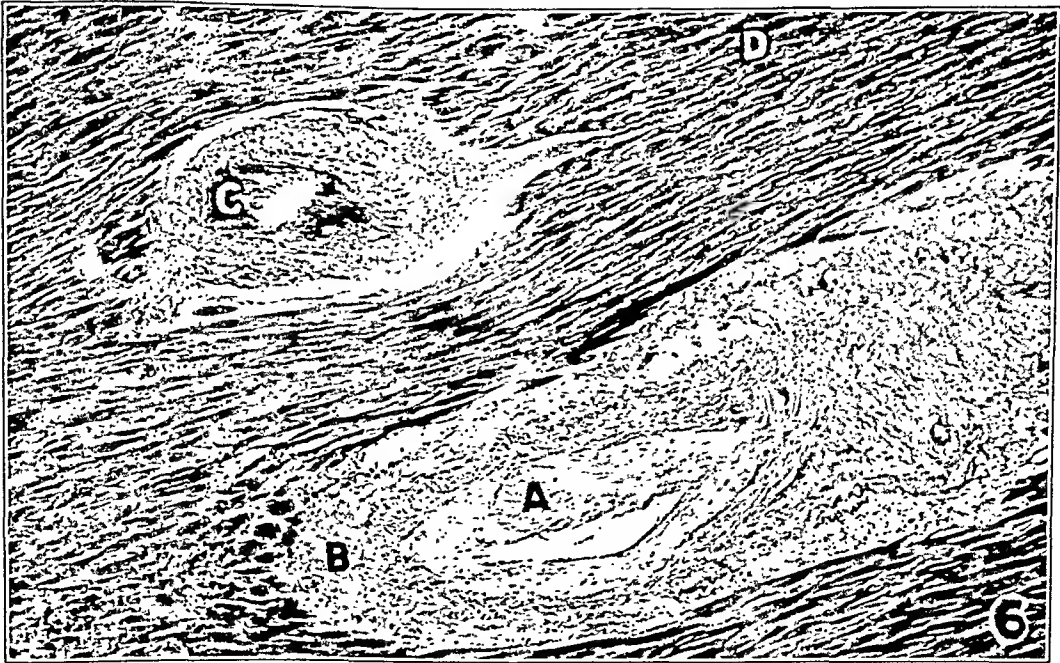
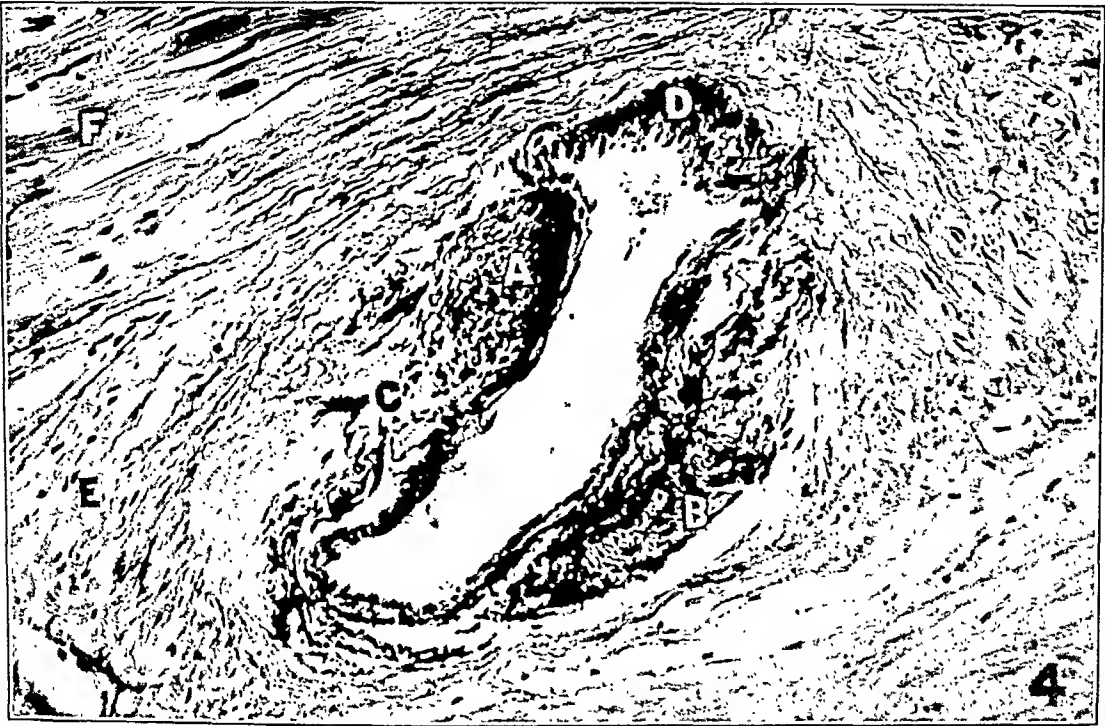


PLATE 34

FIG. 7. Myocardial artery in posterior papillary muscle showing intimal fibrosis superimposed on intimal elastification. Age 36 years. Inactive case. High power. Weigert's elastic and Van Gieson's connective tissue stain.

A = fibrotic zone situated internally to intimal elastified zone; B = intimal elastified zone; C = rather atrophic media; D = myocardium.

FIG. 8. Myocardial artery within the periaortic pericardial sheath showing giant medial hypertrophy with metallaxis. Age 15 years. Active case. Medium power. Weigert's elastic and Van Gieson's connective tissue stain.

A = enormously hypertrophied and markedly edematous media with disorientation of smooth muscle fibers; B = lamella elastica interna; C = elastified adventitia; D = myocardium.

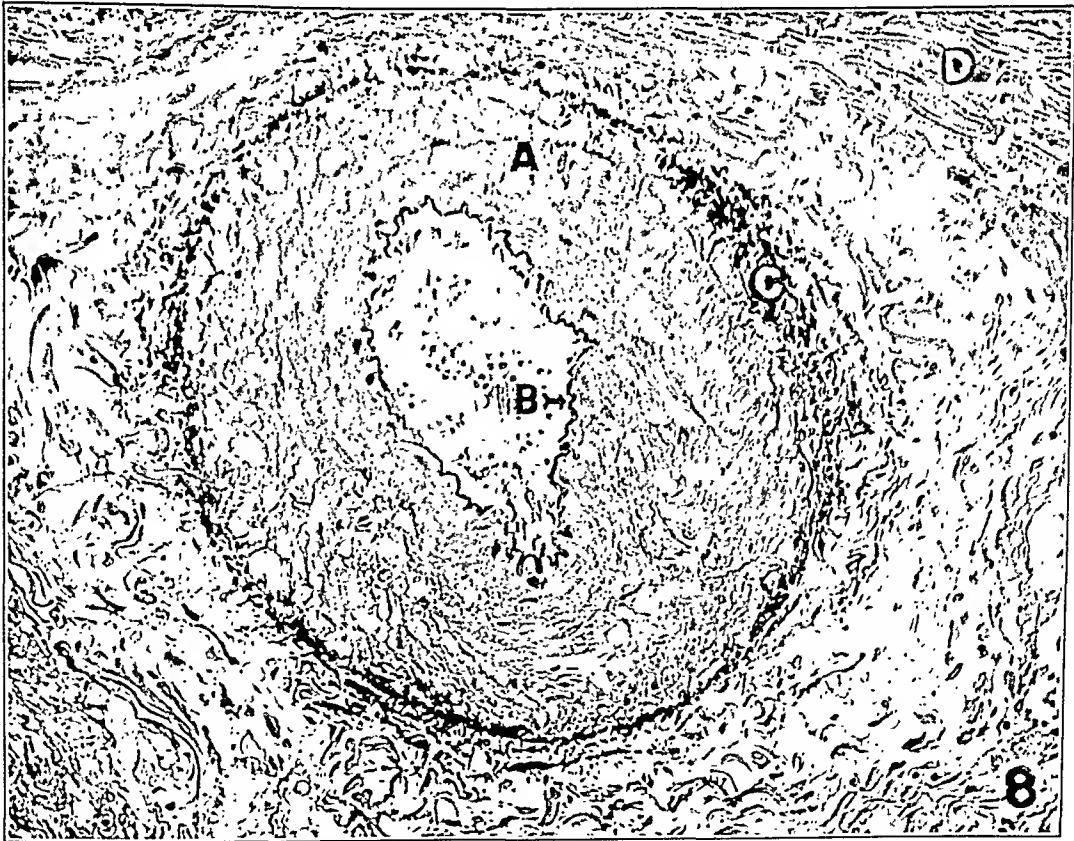
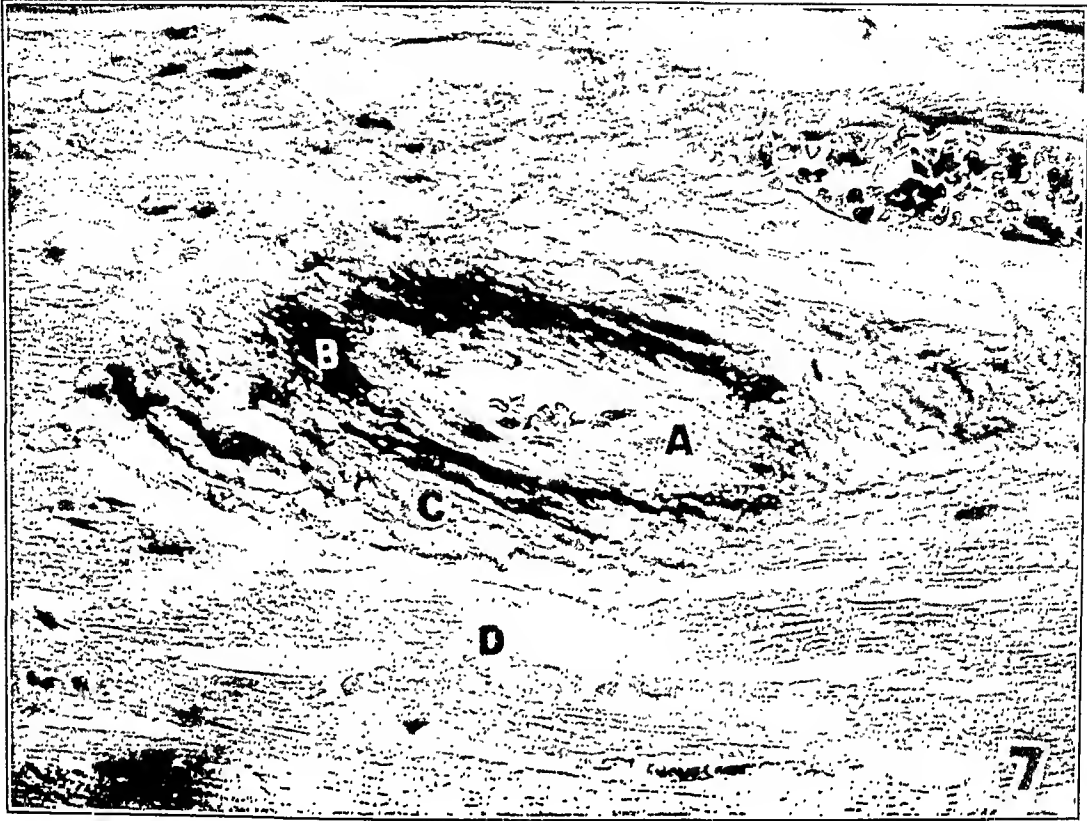


PLATE 35

FIG. 9. Myocardial artery in left ventricle showing exudative and necrotizing panarteritis. Age 25 years. Active case. High power. Hematoxylin and eosin stain.

A = lumen of vessel containing red blood cells and desquamated endothelium; B = necrotic media. Note marked infiltration of adventitia and media with inflammatory cells.

FIG. 10. Myocardial artery in myo-epicardial wedge of right auricle showing subendothelial hemorrhagic arteritis. Age $3\frac{1}{2}$ years. Active case. High power. Hematoxylin and eosin stain.

A = extremely narrowed lumen of vessel containing hyaline thrombus and lined with endothelial cells; B = subendothelial space containing red and white blood cells; C = disrupted media; D = adventitia.

FIG. 11. Myocardial artery in myo-epicardial wedge of right auricle showing net-like fibrinous thrombosis. Age $3\frac{1}{2}$ years. Active case. High power. Hematoxylin and eosin stain.

A = markedly edematous media; B = net-like strands of fibrin producing incomplete occlusion of the vessel; C = adventitia.

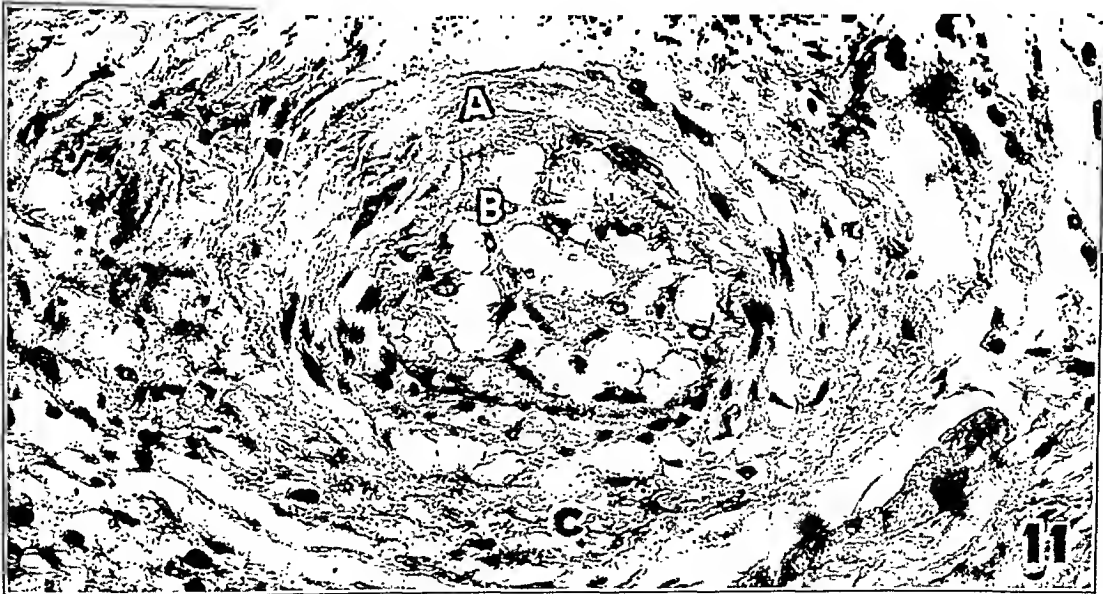
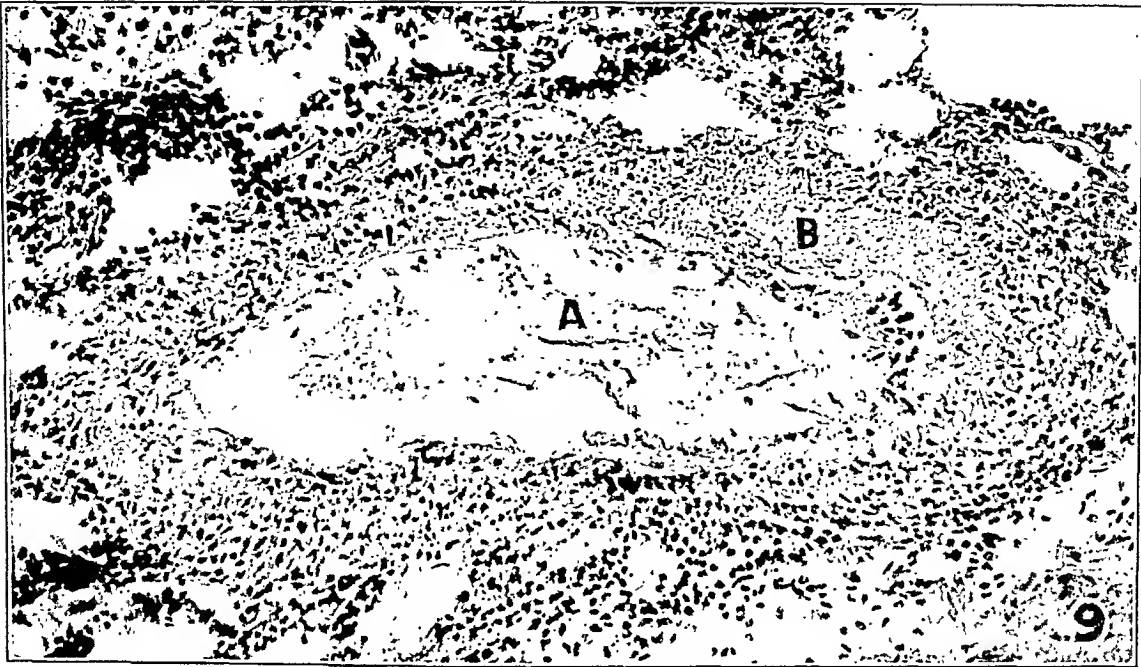


PLATE 36

FIG. 12. Myocardial artery in left ventricle showing endarteritis verrucosa. Age $4\frac{1}{2}$ years. Active case. Medium power. Hematoxylin and eosin stain.
A = typical verrucous lesion; B = remains of media; C = adventitia; D = myocardium.

FIG. 13. Small myocardial arteries in posterior wall of left ventricle showing granular plugged vessels. Age 38 years. Active case. High power. Hematoxylin and eosin stain.
A = typical granular plugged vessel; B = small canalized vessel; C = myocardium.

FIG. 14. Myocardial artery in posterior wall of left ventricle showing canalization. Age 38 years. Active case. High power. Weigert's elastic and Van Gieson's connective tissue stain.
A = obliterated lumen containing three vascular canals; B = elastified adventitia; C = myocardium.

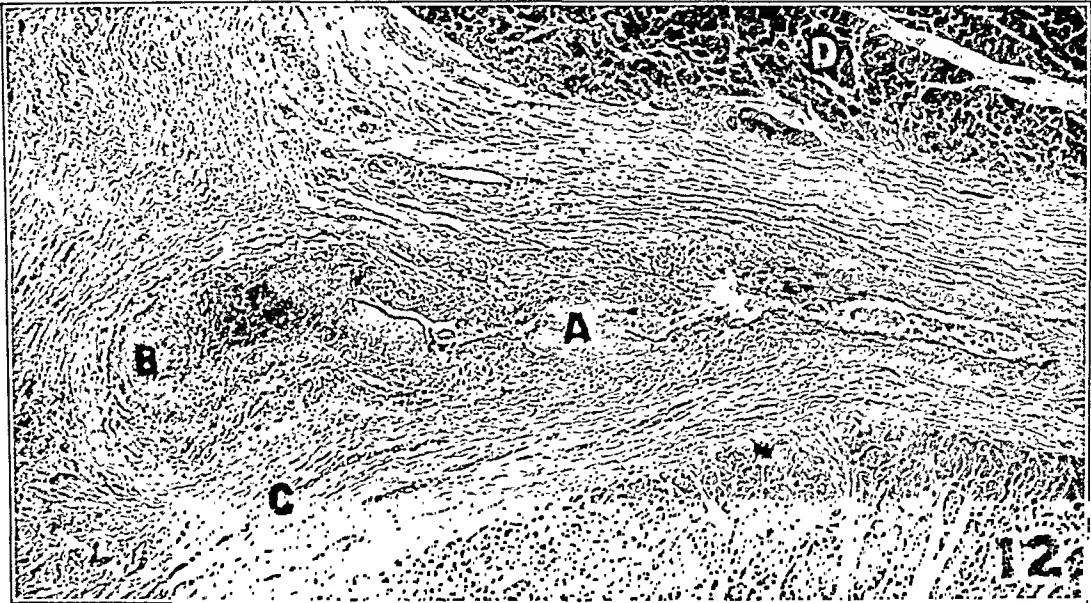


PLATE 37

FIG. 15. Myocardial artery in posterior wall of left ventricle showing endarteritis polyposa. Age 17 months. Active case. High power. Hematoxylin and eosin stain.

A = centrally placed, organizing polypoid mass within the lumen of the vessel; B = media.

FIG. 16. Myocardial artery in the left auricular retro-aortic myo-epicardial wedge showing intimal musculo-elastic hyperplastic lesion (concentric type). Age 13 years. Active case. High power. Weigert's elastic and Van Gieson's connective tissue stain.

A = raised endothelium; B = intimal musculo-elastic hyperplastic layer showing cross-section of longitudinal smooth muscle cells surrounded by mantles of elastic tissue; C = additional inner zone showing early musculo-elastic hyperplastic changes and possibly representing fresh attack; D = markedly edematous media; E = adventitia.

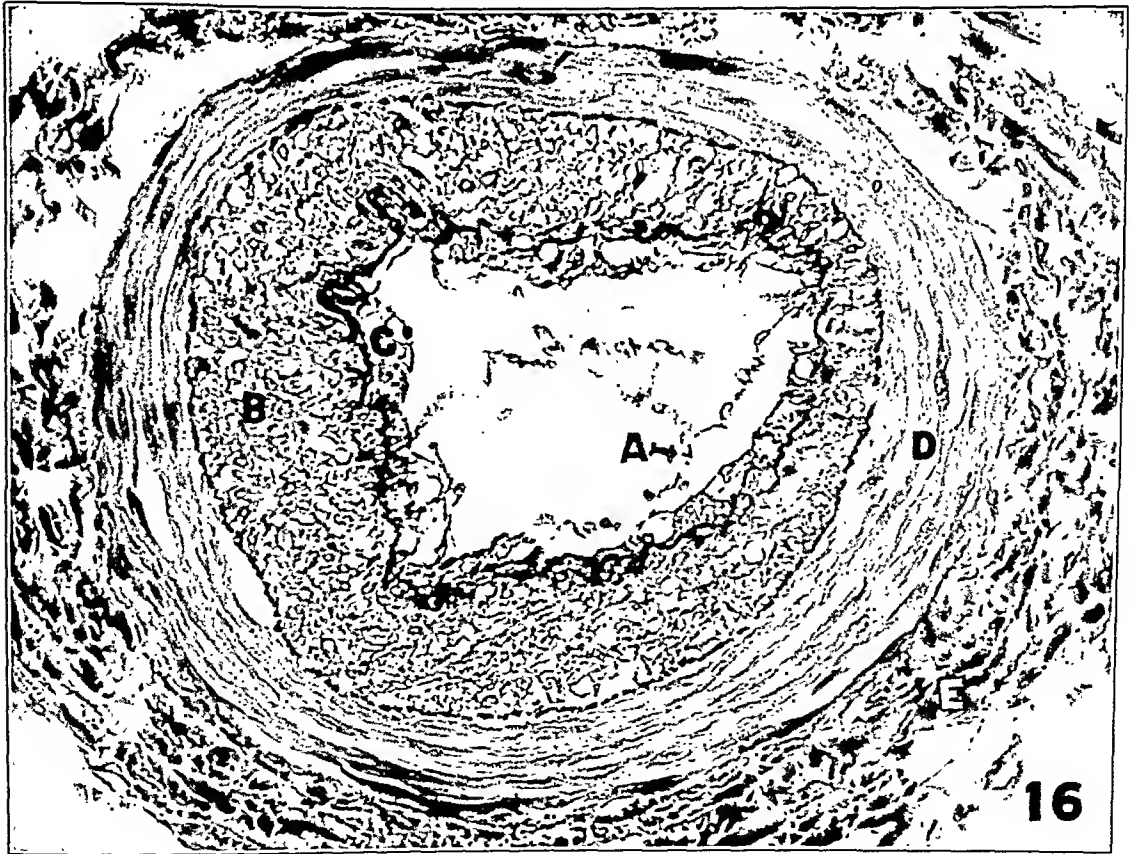
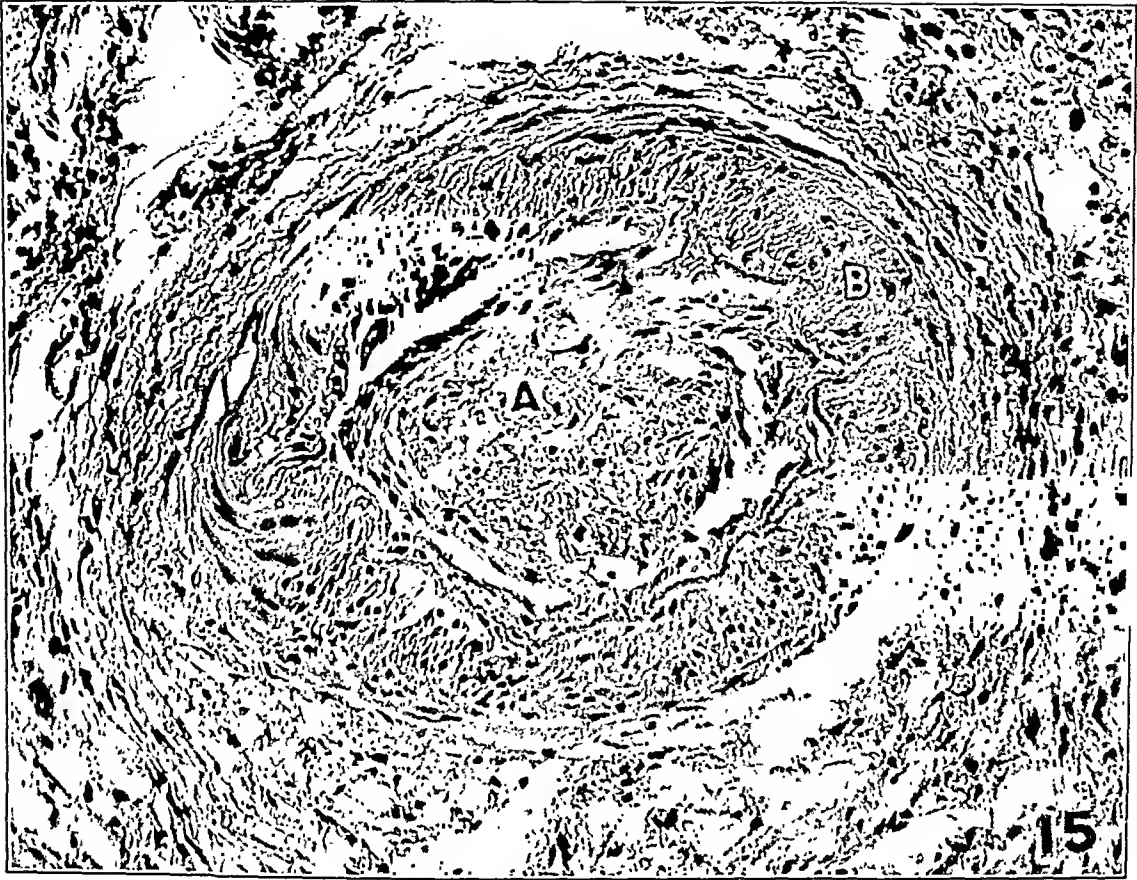


PLATE 38

FIG. 17. Myocardial artery in interventricular septum showing intimal musculo-elastic hyperplastic lesion (eccentric type). Age 11½ years. Active case. Medium power. Weigert's elastic and Van Gieson's connective tissue stain.

A = eccentrically placed, intimal musculo-elastic hyperplastic lesion consisting of longitudinal smooth muscle cells surrounded by elastic mantles. Note somewhat laminated appearance. B = media.

FIG. 18. Myocardial artery in the left auricular retro-aortic myo-epicardial wedge showing end result of intimal musculo-elastic hyperplastic lesion. Age 30 years. Inactive case. Medium power. Weigert's elastic and Van Gieson's connective tissue stain.

A = lumen almost completely obliterated by markedly elastified intima. These elastic fibers, together with collagenous material, have almost completely replaced the longitudinal smooth muscle cells of the intimal musculo-elastic hyperplastic lesion. B = media.

FIG. 19. Myocardial artery in interventricular septum showing intimal musculo-elastic hyperplastic lesion (laminated type). Age 30 years. Inactive case. High power. Weigert's elastic and Van Gieson's connective tissue stain.

A = laminated musculo-elastic hyperplastic lesions, possibly representing different attacks; B = media; C = adventitia.

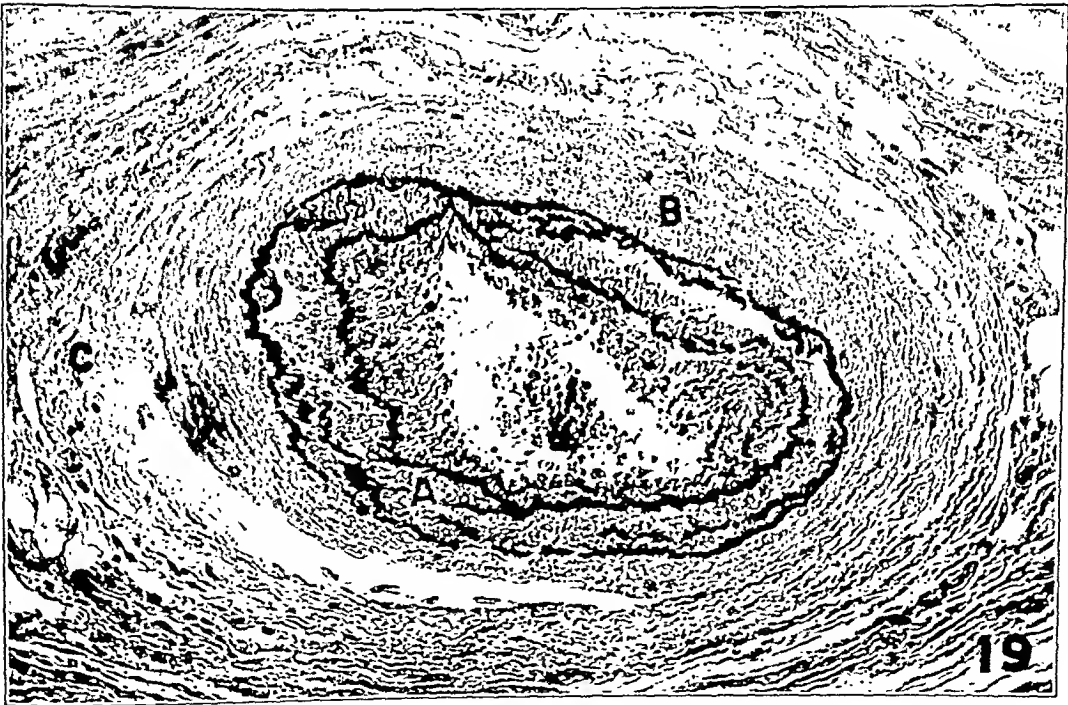
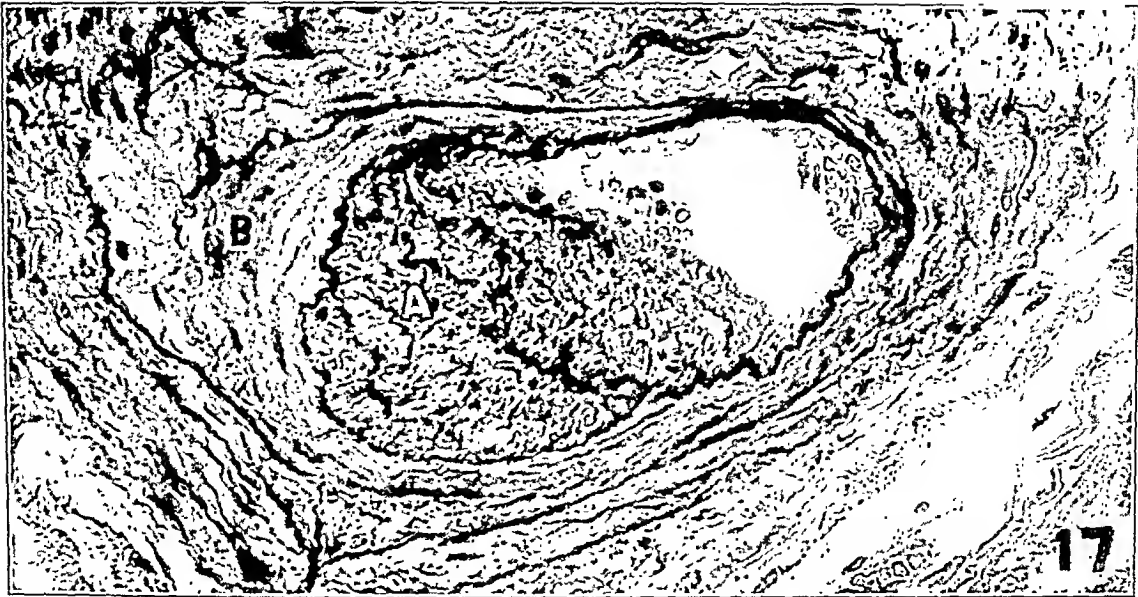


PLATE 39

FIG. 20. A group of vessels situated in the left auricular retro-aortic myo-epicardial wedge showing various characteristic lesions. Age 18 years. Active case. Medium power. Weigert's elastic and Van Gieson's connective tissue stain.

A = vessel showing intimal musculo-elastic hyperplastic lesion; B = vessel showing intimal fibrosis; C = vessel showing intimal fibrosis with some elastification; D = vessel showing giant medial hypertrophy with metallaxis.

FIG. 21. Left anterior descending coronary branch showing scarring and elastification of media. Age 38 years. Inactive case. Medium power. Weigert's elastic and Van Gieson's connective tissue stain.

A = elastified intima; B = distorted, heavily elastified patches in markedly elastified media; C = scarred area in media; D = adventitia.

FIG. 22. Left anterior descending coronary branch showing scarring and elastification of media. Age 50 years. Inactive case. High power. Weigert's elastic and Van Gieson's connective tissue stain.

A = fibrotic layer of intima; B = elastic-hyperplastic layer of intima; C = discontinuities in lamella elastica interna; D = heavily elastified media with splitting and fraying of elastic membranes; E = conspicuous scar in media with replacement of smooth muscle by connective tissue; F = adventitia containing scattered lymphocytes.

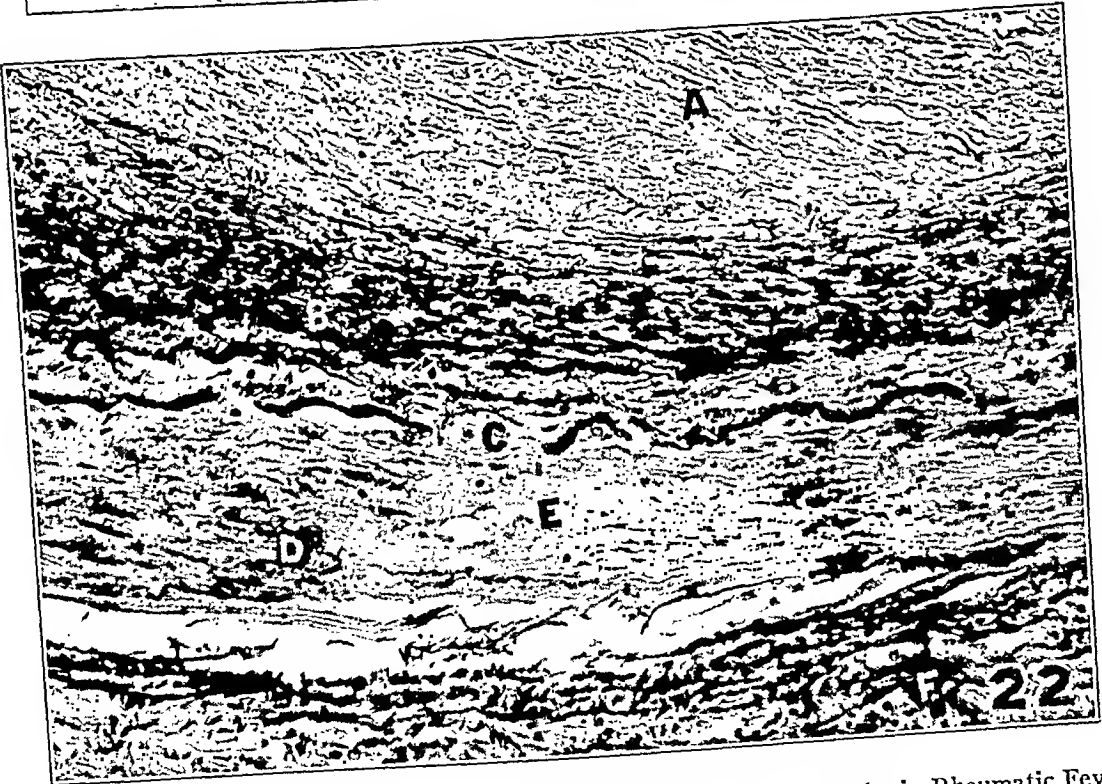
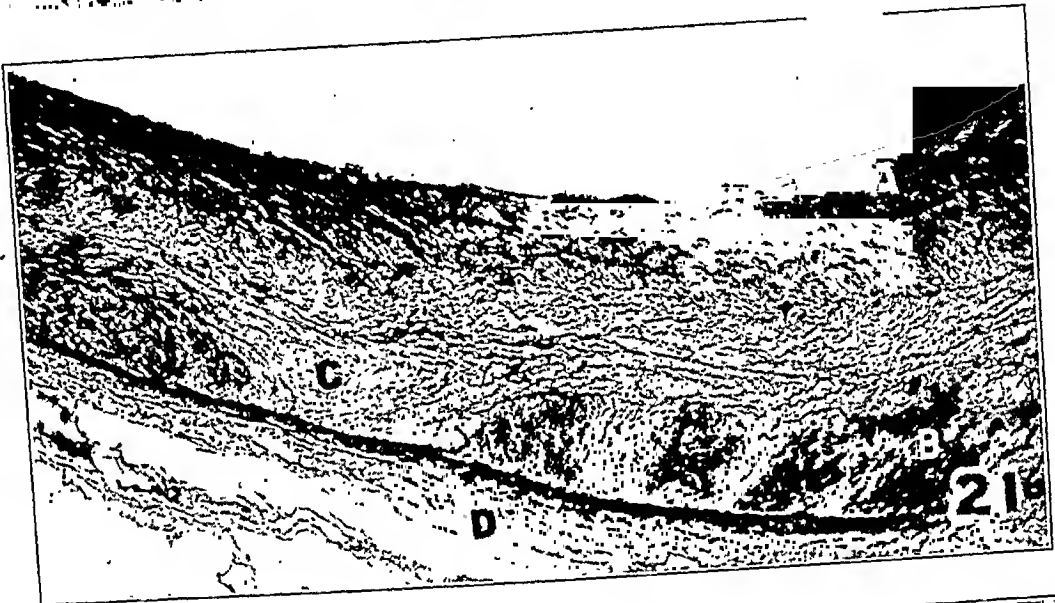
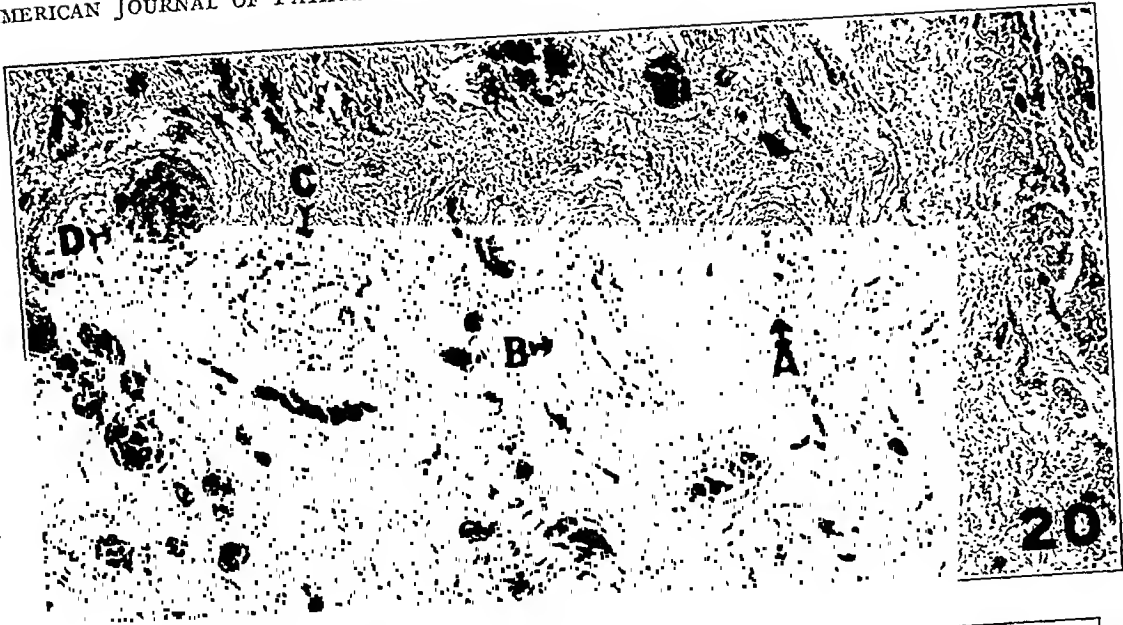


PLATE 40

FIG. 23. Right circumflex coronary artery showing fibrotic intima and edematous media. Age 14 years. Active case. Medium power. Hematoxylin and eosin stain.

A = fibrotic thickened intima; B = marked edema of media; C = adventitia.

FIG. 24. Right circumflex coronary artery showing inflamed edematous media and intimal reduplication. Age $4\frac{1}{2}$ years. Active case. Medium power. Weigert's elastic and Van Gieson's connective tissue stain.

A = normal portion of media showing compact smooth muscle; B = swollen and edematous portion of media with inflammatory cells; C = recently formed reduplicated layer; D = adventitia.

FIG. 25. Left anterior descending coronary branch showing edema of media and dissolution of lamella elastica interna. Age $3\frac{1}{2}$ years. Active case. Medium power. Weigert's elastic and Van Gieson's connective tissue stain.

A = disappearance of lamella elastica interna; B = edematous media with swelling and prominence of medial cells; C = adventitia; D = myocardium.

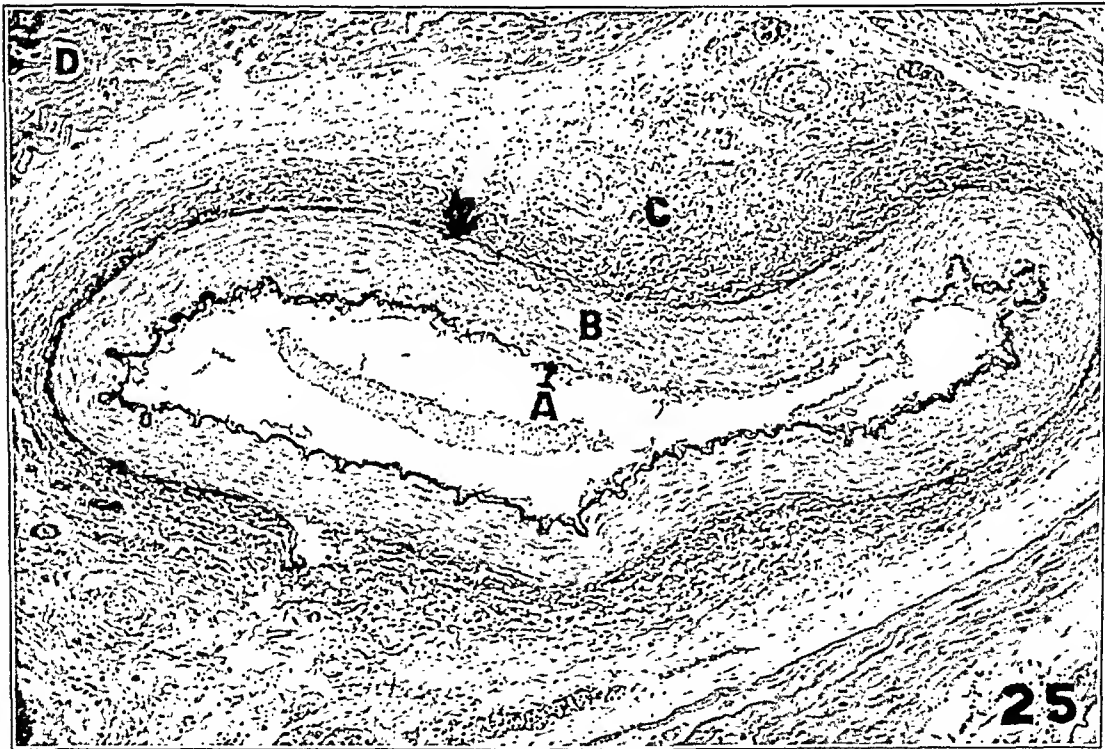
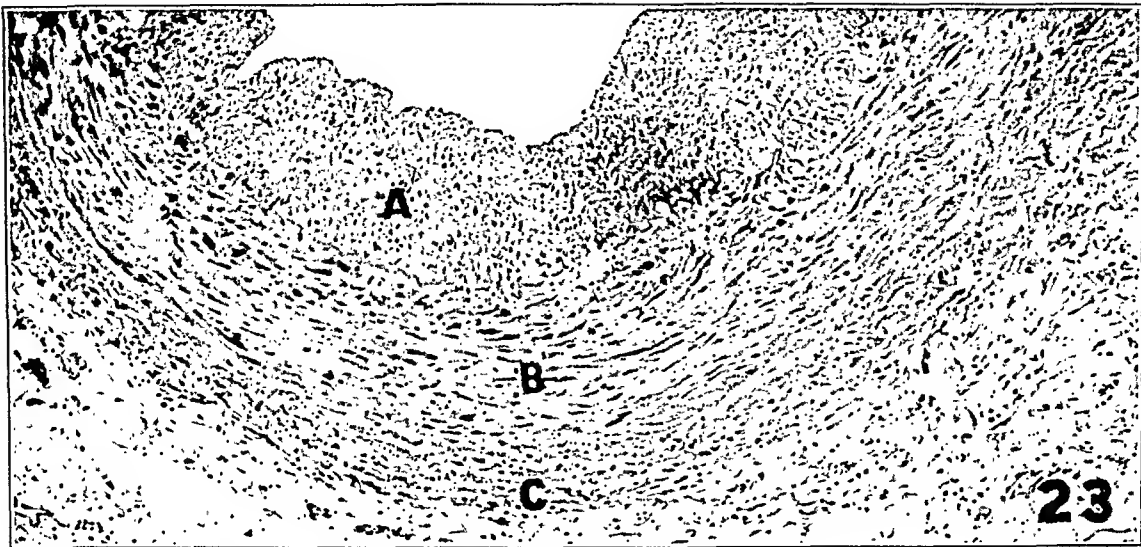


PLATE 41

FIG. 26. Left circumflex coronary artery showing necrotizing arteritis in case of periarteritis nodosa with rheumatic fever. Age $7\frac{1}{2}$ years. Medium power. Weigert's elastic and Van Gieson's connective tissue stain.

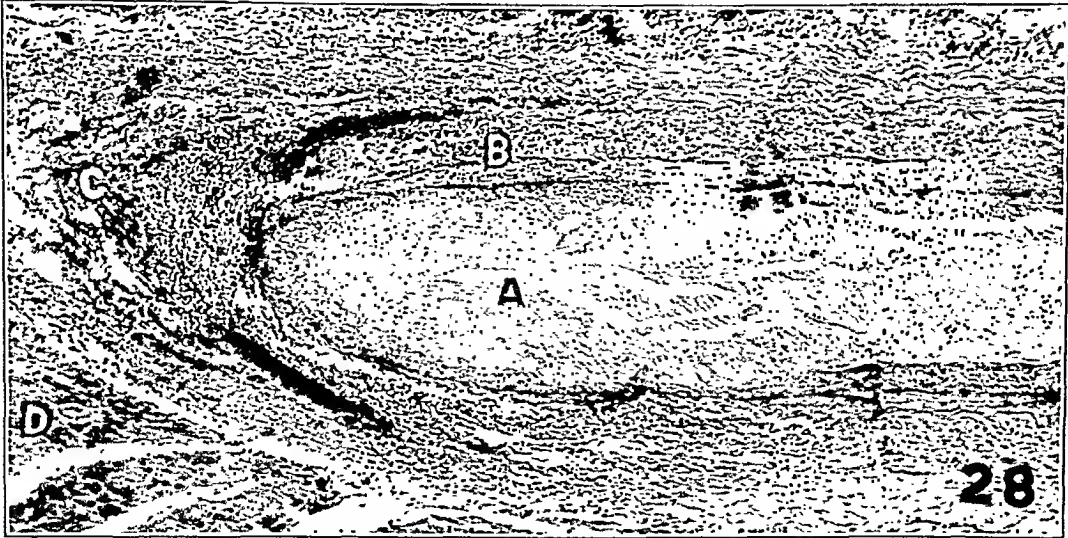
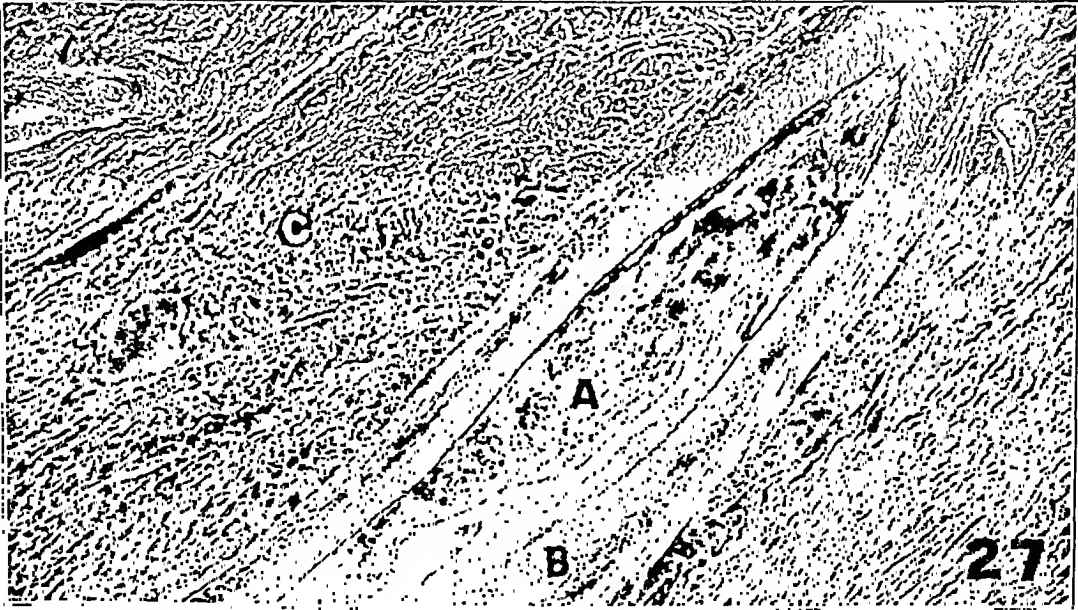
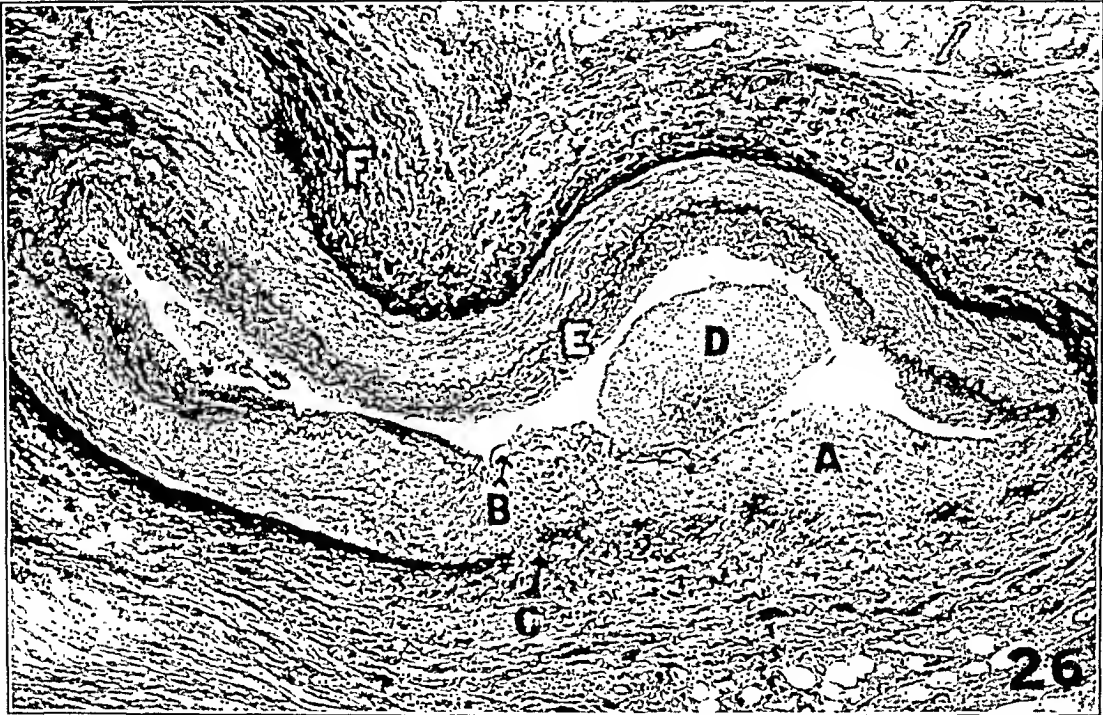
A = markedly edematous and inflamed media; B = rupture and dissolution of intimal elastic membranes; C = rupture, fraying and dissolution of adventitial elastic membranes; D = blood-platelet thrombus; E = elastic-hyperplastic intima; F = adventitia.

FIG. 27. Thrombosis of left circumflex coronary artery. Age 17 months. Active case. Medium power. Weigert's elastic and Van Gieson's connective tissue stain.

A = lumen of vessel almost completely occluded by blood-platelet thrombus; B = media; C = myocardium.

FIG. 28. Left circumflex coronary artery showing arteritis and occluding granular plugged lesion. Age 25 years. Active case. Medium power. Weigert's elastic and Van Gieson's connective tissue stain.

A = granular plugged lesion occluding lumen; B = edematous inflamed media; C = markedly inflamed adventitia; D = myocardium.



ENDOMETRIOSIS OF THE UMBILICUS *

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At the meeting of the American Association of Pathologists and Bacteriologists in Rochester, New York, in 1927, I described 2 cases of endometriosis of the umbilicus under the title of "Menstruating Umbilical Tumors." Several authors have since included these 2 cases in lists of the reported cases of this interesting condition, depending upon a brief abstract ¹ for their information. In order to make more complete data available to others, these cases are now published in full for the first time.

In 1927, endometriosis of the umbilicus, or umbilical adenomyoma, as it was then more commonly called, was considered a very rare condition. About 35 cases had been described. In the next few years reports of this condition were numerous, so that by 1931 Spitz ² designated his case as the 55th. There is no occasion to review the literature of these many cases at the present time. Spitz ² has provided a comprehensive tabulation and Enzer, ³ in 1930, again brought the literature up to that date and discussed some of the more important questions that arise in connection with umbilical endometriosis.

Those who are interested in as complete a compilation as possible may add the following cases which are not referred to by either Spitz ² or Enzer ³: Terrier ⁴ (1887), Tourneux ⁵ (1889), Rohdenburg ⁶ (1919), Cullen ⁷ (1920) 2 cases, Walz ⁸ (1926), Butomo and Schereschewsky ⁹ (1930), Habbe ¹⁰ (1931), Thompson ¹¹ (1931), Stetson and Moran ¹² (1932), Siedentopf ¹³ (1932), Longwood ¹⁴ (1932), Herberz ¹⁵ (1933) 3 cases, and Lanos and Busser ¹⁶ (1933).

Our own cases, 2 in number, are as follows.

CASE REPORTS

CASE I (2783-V). On May 18, 1918, there was delivered to the laboratory by an outside physician a mass which was said to be an

* Received for publication August 23, 1934.

umbilical tumor. It had been excised from a woman about 45 years of age. No history was obtainable other than that the patient had no children, although married for more than 15 years. A diagnosis of congenital umbilical cystadenoma was made and a brief description of the histological features was recorded.

After receiving the second case a number of years later the material from the first case was restudied. The gross material consisted of the umbilicus surrounded by an elliptical zone of skin. Centered beneath the umbilicus there was a firm nodular mass about 2 cm. in diameter. On section this was found to contain small spaces, the largest about 2 mm. in diameter. There was no evidence of hemorrhage in the interior of the mass.

Microscopic examination showed that the specimen was covered by skin on one side and by peritoneum on the other. The small cystic spaces, more than twenty of which appeared in a single section, occurred in a non-encapsulated mass which occupied almost the entire thickness of the wall. Over it, both corium and epidermis were intact. The cysts, of varying size and shape, were lined by a columnar epithelium which frequently showed cilia (Fig. 1). About this, in certain examples only, there was a richly nucleated and finely reticular stroma ("cytogenic stroma"), resembling the interglandular tissue of endometrium. In the largest cyst there were many phagocytes containing old blood pigment and a mass of well hemolyzed red blood cells. Outside of the cellular stroma, or immediately about those cysts around which such stroma was missing, there was fibrous connective tissue of varying density, some of which showed the usual appearance of the remains of the umbilical scar. There was no definite encapsulation of the mass but this somewhat atypical connective tissue passed rather abruptly into the normal stroma of the region. There was no involuntary muscle present other than that of the blood vessels. This opinion, based originally on routine hemalum and eosin stains, was confirmed by subsequent staining with Van Gieson's mixture. The large sweat glands of the region occurred near, but not within, the nodular mass.

The gross and microscopic features were thus characteristic in every respect of endometriosis of the umbilicus.

CASE 2 (2788-AE). An American housewife, 49 years of age, was referred to the general surgical service from the out-patient department with a diagnosis of questionable melanoma of the um-

bilicus. The patient had been married twice. She had had six children by her first husband, none by the second. About $2\frac{1}{2}$ years before coming to the hospital she had noticed a small painful swelling in the umbilicus. This was not preceded by trauma nor could she recall having had a mole at that site. The tumor had slowly increased in size and the patient stated that there had been some oozing of blood at the time of the menstrual periods only. Those who first examined her were inclined to discredit this assertion, but with the appearance of the menses while she was still in the hospital such oozing of blood did occur.

Examination of the patient showed at the umbilicus two protruding nodules, each about 3.5 cm. in diameter. These were separated by a vertical fissure in the midline. The mass to the left was hard and its lower part was bluish black in color. The right portion was softer and pigmented but slightly. The umbilicus, with these nodules and a zone of skin, was excised with the endotherm knife. The peritoneal cavity was opened and a small portion of the preperitoneal fat removed with the mass.

On microscopic examination the greater part of each nodular mass was found to be made up of cystic structures of varying size, with cubical to columnar epithelium, the latter often ciliated (Fig. 2). Practically every one of these spaces had about its epithelial lining an investment of a cellular reticulum, exactly like endometrial stroma in a highly cellular phase. In many of the spaces there was recent hemorrhage, showing but little hemolysis. In others there were phagocytes containing older blood pigment. Outside of the zones of "cytogenic stroma" there was an abundant dense connective tissue such as is normally found at the umbilicus. There was no demonstrable connection between the peritoneum and the structures described. The epidermis was lacking, however, over a portion of the surface and here there was shallow ulceration and a secondary inflammatory reaction. Sweat glands were abundant and were to some extent intermingled with the cystic structures, but there was no evidence of any connection between the two. Smooth muscle was entirely lacking.

DISCUSSION

In addition to placing these 2 cases of umbilical endometriosis on record, it is desired to call attention to the fact that the propo-

nents of those theories of the origin of endometriosis that require implantation or metastasis of fragments of endometrium are confronted with serious difficulties in explaining its occurrence in this situation. Jacobsen ¹⁷ has stated that he believes that there are present in these cases small umbilical hernial sacs into which endometrial tissue that has been set free in the peritoneal cavity has found its way. While endometriosis has been described in connection with umbilical hernia (Mintz ¹⁸), in most of the reported cases, as in our own, no evidence of umbilical hernia has been found. Also, if this is the explanation, it is surprising that endometriosis has never been described for any other peritoneal recess of the upper half of the abdomen, including the lesser peritoneal sac. Since at the umbilicus there is opportunity for a physiological inclusion of peritoneal mesothelium and subserosa, the advocates of the serosal theory of endometriosis have no difficulty with this localization, and endometriosis should have a single explanation no matter where it occurs.

CONCLUSIONS

In conclusion, attention is invited particularly to the following points.

1. With over 70 cases described in medical literature the umbilicus can no longer be considered a rare location for endometriosis.
2. Smooth muscle is commonly not present in endometriosis of the umbilicus and is not an essential element in these formations. The term "adenomyoma" is altogether inappropriate.
3. The sweat glands, which have been mentioned repeatedly in connection with umbilical endometriosis, have no part in the genesis of this condition.
4. The origin of umbilical endometriosis is explainable only with the greatest difficulty under the implantation and metastasis theories, but is readily understandable under the various modifications of the serosal theory.

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DESCRIPTION OF PLATE

PLATE 42

- FIG. 1. Case 1. Umbilical endometriosis. Ciliated columnar epithelium surrounded by a richly nucleated spindle-celled stroma. This in turn is embedded in a dense connective tissue without smooth muscle. Hemalum and eosin stain. $\times 156$.
- FIG. 2. Case 2. Umbilical endometriosis. A menstruating umbilical tumor. Structure is the same as in Case 1. In addition some of the gland spaces show recent hemorrhage. $\times 156$.



I



2

THE ECTOPIC DECIDUAL REACTION AND ITS SIGNIFICANCE IN ENDOMETRIOSIS *

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Almost all pathologists whose duties include the routine examination of a large amount of surgical material are familiar with the nodular or plaque-like decidual reaction of the subserosa which not infrequently makes possible the diagnosis of pregnancy from an appendix. This condition was first described for the pelvic peritoneum in 1887 by Walker¹ in connection with extrauterine pregnancy. His findings were confirmed by numerous observers, some of whom found a serosal decidual reaction to be present in intrauterine pregnancy as well. The area in which this change was known to occur was enlarged during this period by finding it upon the ovary, oviduct, broad ligament, anterior rectal wall, small intestine where adherent in the pelvis, and the omentum where adherent to an oviduct in which there was an ectopic gestation. The literature of this phase was reviewed by Hirschberg² in 1905, who then described for the first time the decidual reaction upon the appendix. This observation has been confirmed repeatedly, until now knowledge of it has become a necessary part of the armamentarium of the pathologist. In an active diagnostic service several examples may be seen in a single year.

Upon the appendix the decidual reaction appears as small nodules, streaks and patches produced by a thickening of the serosa or, more properly, the subserosa. These nodules are frequently mistaken by the surgeon for tubercles, metastatic neoplasm or organizing exudate. The microscopic appearance is characteristic and may be taken as a type form for this reaction wherever found (Figs. 1 and 2). Beneath an intact mesothelium and apparently developing from the stroma cells of the subserosa, nests of large cells with slightly basophilic cytoplasm and spherical, usually eccentric, nuclei occur. These large cells, often 30 microns or more in diameter, resemble in

* Received for publication August 23, 1934.

every respect the decidual cells of endometrial origin. Geipel³ has shown that, like intrauterine decidual cells, the peritoneal counterpart is often richly supplied with glycogen.

After Hirschberg, there were further significant extensions of the area in which the serosal decidual reaction was known to occur. The literature of the intervening period is reviewed by Rosenberger.⁴ Attention is directed particularly to the demonstration of the ectopic decidual reaction in pelvic lymph nodes by Geipel.⁵ In some of the same lymph nodes duct-like or gland-like spaces lined by cubical or low columnar epithelium were found. At the present time such areas would be interpreted, in all probability, as endometriosis with a secondary decidual reaction. In a later paper Geipel³ reported the occurrence of the decidual reaction upon the diaphragm and the splenic capsule.

Decidual reaction in the cervical mucosa has been observed repeatedly. It is especially apt to occur with placenta previa. Róna⁶ has described a decidual reaction at the external os; and the development of such an excessive decidual formation in the vaginal wall as to merit the name of *polyposis vaginæ* has been reported by Zacherl.⁷ While his excellent illustrations show glands of cervical type to be present, those glands about which the decidual reaction occurs are of a different type and the entire complex suggests a decidual reaction taking place in an area of endometriosis. Yet Zacherl believed that endometriosis could be excluded.

In an especially uncertain position is the question of a possible ectopic decidual reaction without either intra- or extrauterine pregnancy. In all the examples to which reference has been made above, pregnancy was present. But Schiller⁸ and Schereschewsky⁹ have shown the development of decidual formations in the apparent absence of pregnancy. Whether this is another manifestation of *decidua menstrualis* and is comparable to the premenstrual changes in the endometrium, or whether the fact of pregnancy escaped detection in the reported cases, cannot be determined positively at the present time.

Endometriosis has been described for the uterine wall, oviduct, ovary, mesovarium, broad ligament, round ligament, vagina, cervix, bladder wall, rectum, sigmoid colon, cecum, mesocolon, appendix, small intestine, retroperitoneal and inguinal lymph nodes, umbilicus and laparotomy scars (*Lit.* by Jacobsen,¹⁰ Polster¹¹). It will at once

be noted that, with few exceptions, the regional distribution of endometriosis coincides with that of the ectopic decidual reaction. The only significant difference is in the reported occurrence of a decidual reaction upon the diaphragm and the splenic capsule. Apparently, endometriosis has never been noted as occurring above the level of the umbilicus. It is possible to go even farther in the comparison, for there is a similarity in the relative frequency of occurrence of the two conditions in various regions. Polster found in a collection which included over 1000 cases of endometriosis of the ovaries, tubes and uterine ligaments, 90 in the rectovaginal septum, 80 in the intestinal tract, 56 in scars, 34 in the inguinal regions and 30 at the umbilicus. In a general way, the incidence decreases as the distance from the ovaries increases, as is true of the ectopic decidual reaction. If the analogy from distribution of the two conditions is as significant as it appears to be, the very rare occurrence of the, as yet, unknown endometriosis of the diaphragm may be predicted.

In addition to the similarity in regional distribution between endometriosis and ectopic decidual formation, which was noted as early as 1925 by Schiffman and Seyfert,¹² there are other significant features. Geipel^{3, 5} found that glandular spaces were frequently present in the retroperitoneal nodes showing an ectopic decidual reaction. Conversely, a decidual reaction has been described for the "cytogenic" stroma of endometriosis in almost every location, including umbilicus, cervix and vagina. The "cytogenic" stroma of endometriosis is the interglandular stroma of the endometrium. They function alike in the development of decidua. But, under appropriate hormonal stimulation, exactly similar decidual cells may develop from the cellular elements (apparently of the connective tissue group) which lie immediately beneath the serosal cells proper over a wide area in the lower abdomen and pelvis.

Such similarities and identities of morphology and function support the serosal theory of origin of endometriosis. The subserous stromal cells must possess pluripotentiality in differentiation. Under the influence of suitable but dissimilar stimulation either the "cytogenic" stroma of endometriosis or the decidual reaction may result. If the former develops, it in turn may subsequently be induced to become decidual. In the decidual reaction the mesothelial cells do not appear to take any part. They lie apparently unchanged on the

masses of decidual cells. While not properly germane to the subject of this paper, it must be assumed that the surface mesothelial cells likewise possess latent potentiality in differentiation and that from them, particularly when entrapped in adhesions or in scar tissue, and again under stimulus of a hormone, the epithelial elements in endometriosis are derived.

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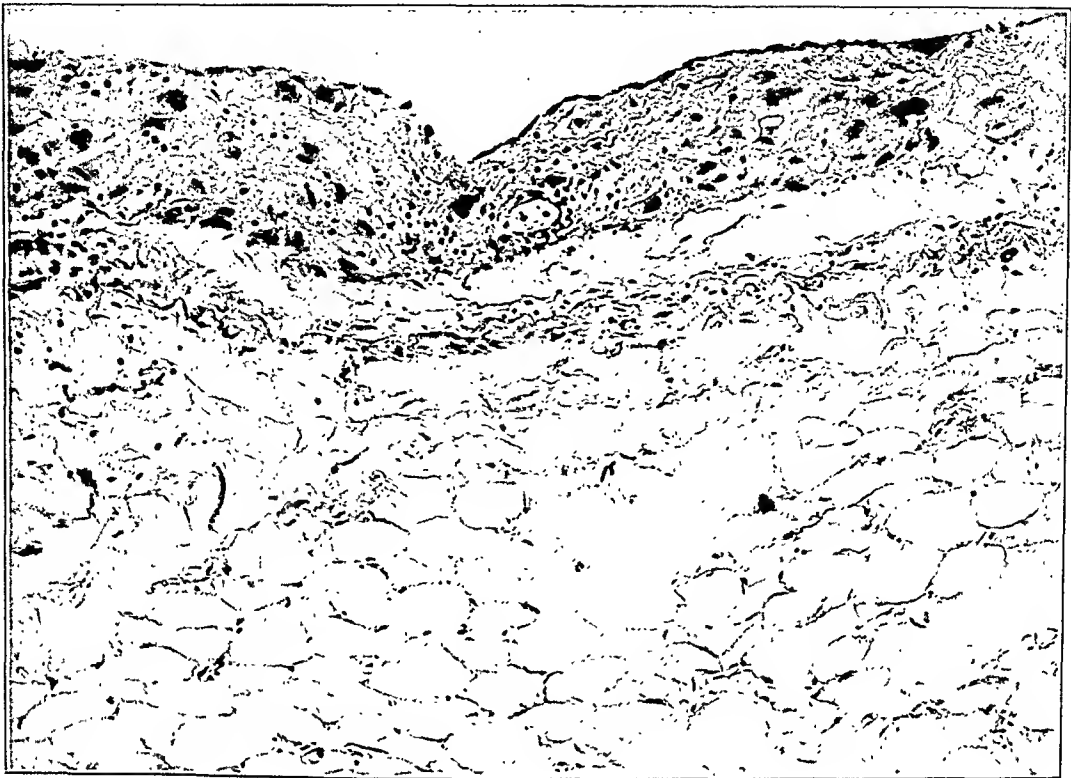
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DESCRIPTION OF PLATE

PLATE 43

FIG. 1. Decidual reaction in the subserosa of the meso-appendix, in the fifth month of pregnancy. Hemalum and eosin stain. $\times 198$.

FIG. 2. Decidual reaction in the subserosa of the appendix, from the same case as the preceding. $\times 310$.



1



2

DIFFUSE ARTERITIS OF SYPHILITIC ORIGIN *

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That the *Treponema pallidum* may cause arteritis has long been recognized. The aorta and cerebral vessels are involved most frequently, although lesions in small vessels elsewhere, such as those of the extremities, have been found occasionally. This subject was reviewed recently by Connor¹ in his Billing's lecture entitled "Development of Knowledge Concerning Role of Syphilis in Cardiovascular Disease." In this lecture Connor noted how infrequently organisms have been found in the lesion.

Since we have had an opportunity to study both clinically and at autopsy a case with widespread involvement of the smaller arteries, and since the *Treponema pallidum* has been identified in one of these vascular lesions, it seemed wise to report our findings in some detail.

REPORT OF CASE

Clinical History: The patient, a 26 year old male, was admitted for the first time to the hospital in January, 1933. At that time he complained of a painful sore on the penis of 7 days duration and of a urethral discharge. His past history was irrelevant, except that he had had gonorrhoea 6 years previously. He stated that he usually went on an alcoholic debauch monthly for 24 hours, at which time he was exposed sexually. The last occasion was 6 weeks previously.

Examination revealed a normal male, except for a chancre 1.7 by 1 cm. on the penis just back of the corona, and the presence of a moderately profuse urethral discharge. The discharge contained numerous organisms typical of the gonococcus. Scrapings from the ulcer, when examined by the dark field method, contained numerous treponemata. The patient remained in the hospital for 14 days, during which time he received five intravenous injections of arsphenamine. The dosage ranged from 0.1 gm. to 0.4 gm.

Following the last injection the patient had an erythematous dermatitis, due presumably to the arsphenamine medication. From this time until Aug. 18, 1933, the patient received three series each of arsphenamine and mercury succinimide in our Out-Patient Department. Hinton and Wassermann tests were repeatedly negative after April 4, 1933. He was treated for urethritis in the Genito-Urinary Department. On August 21, 1933, he was found to have a bilateral epididymitis. The patient was not seen again until Oct. 30, 1933, when

* Received for publication August 14, 1934.

he returned complaining of pain in the left calf and foot. This pain was shooting in nature and aggravated by walking. Examination at this time revealed no objective evidence of disease, such as swelling, redness or tenderness.

He was seen again on Nov. 15, 1933, with the complaint that the pain in his foot had persisted. He stated also that he had lost weight, had a poor appetite and was constipated. In January the patient weighed 188 pounds; his weight now was 153 pounds. Examination again revealed nothing abnormal, except for evidence of loss of weight and a chronic urethritis. His blood pressure was not recorded at this time. A roentgenogram of the left foot revealed slight atrophy of bones but nothing otherwise abnormal.

He was readmitted to the hospital as an emergency case on Dec. 4, 1933. Because of his confused, irrational state the interval history had to be obtained from his sister. The latter stated that the patient had had severe headaches for 1 week for which he had taken numerous "anacin" tablets and that he had taken twenty-eight tablets the day previously. Each "anacin" tablet contained three grains of acetophenetidin. Headache had persisted in spite of medication, and he had become very irritable and nervous. The morning of admission his vision became involved so that he could distinguish light from dark only.

Physical examination was unsatisfactory on account of the irritability and uncoöperative attitude of the patient. His blood pressure was 230 mm. mercury systolic and 100 mm. diastolic, whereas in January, 1933, it was 110/75 mm. of mercury. The heart seemed normal in all respects, and the walls of the peripheral vessels were not thickened. He was able to count fingers correctly at four feet. Although ophthalmoscopic examination was not satisfactory, no abnormality could be found other than a questionable blurring of the disc margins. All neurological reflexes were normal. The temperature was 100° F. and white blood cell count 12,000 per cmm.

Lumbar puncture at this time revealed a clear, colorless fluid under increased pressure. The fluid contained thirty lymphocytes and nine polymorphonuclear leukocytes per cmm. and an increased amount of globulin. Wassermann reactions on both blood and spinal fluid were negative.

Forty-eight hours after admission the patient seemed normal except for a slight persistent headache. Another lumbar puncture revealed only slight increase of pressure. The fluid at this time contained four lymphocytes per cmm. and a normal amount of globulin. The blood pressure had fallen gradually to 145 mm. mercury systolic and 98 mm. diastolic.

The patient's condition seemed to improve until Dec. 11, 1933, when he complained of cramp-like pain across the upper abdomen. This was accompanied by hiccough and periodic vomiting. Although tender to pressure there was no rigidity of the abdominal wall. At this time the white blood count showed 39,500 leukocytes per cmm. He was seen by Dr. Cutler, who could find no indication for surgical intervention. A stool, which had been free of blood previously, now contained blood in visible amount. The explanation for this was uncertain as the patient had internal hemorrhoids as a possible source. The red blood cell count and hemoglobin were normal and blood urea nitrogen was 21 mg. per cent. Another lumbar puncture was performed, which showed a considerable increase in pressure but nothing otherwise abnormal. With the persistence of nausea, vomiting and hiccough his condition grew worse gradually so that by Dec. 18, 1933, he was in a semicomatose state. At this time there was definite edema of the optic nerve heads, numerous areas of

hemorrhage in the right fundus oculi and incontinence of urine and feces. In view of the possibility of an intracranial tumor he was transferred to the surgical service where ventriculography and encephalography were performed with inconclusive results. His condition failed to improve and he died on Dec. 21, 1933. During the final 10 days of his illness seven white blood cell counts were done. The values of these ranged from 20,000 to 39,500 cells per cmm. His temperature at no time was above 100° F.

In view of the protean manifestations, a hypertension of rapid onset, hemorrhages into the retina, a probable mesenteric thrombosis, pains of unexplained etiology in an extremity, and a marked leukocytosis with little or no febrile response, the clinical diagnosis of vascular disease with multiple thromboses was made.

AUTOPSY REPORT

Autopsy was performed 2 hours after death with the following findings.

Body: The body was that of a moderately well developed and well nourished white male, 172 cm. in length. Pitting edema of the lower extremities was present. There was a bilateral external strabismus. A small atrophic scar of the penis was found at the site where the primary chancre had been.

Peritoneal Cavity: The important findings will be considered in a discussion of the alimentary tract.

Pleural Cavities and Mediastinum: These regions were normal except for fibrous adhesions between the apex of the right lung and the parietal pleura.

Heart: The pericardial cavity was normal. The heart weighed 300 gm. There was a patent foramen ovale, which was 5 mm. in diameter. The four valves, myocardium and arteries were normal.

Lungs: The right lung weighed 700 gm. and the left 630 gm. In the lower lobe of each lung there were scattered and confluent, gray, nodular areas of pneumonic consolidation. Sanguinopurulent exudate could be expressed from these regions. The mucosa of the primary and secondary bronchi showed irregular areas of superficial necrosis covered by a grayish yellow fibrinopurulent exudate. There were no infarcts. The arteries and veins were normal.

Celiac Axis and Branches: The splenic, hepatic and left gastric arteries and their major branches appeared to have normal walls and lumens. The small branches of these major trunks will be described in connection with the organs which they supplied.

Spleen: The spleen weighed 300 gm. A few fibrous adhesions

were attached to the capsule. The pulp, trabecular structure and vessels were normal.

Pancreas: The pancreas weighed 110 gm. There were small indistinct areas of lobular fibrosis. Small arteries were prominent because of their thick, firm walls and very narrow lumens, several of which appeared to be occluded.

Liver and Gall-Bladder: The liver weighed 1800 gm. In the left lobe along the anterior border a subcapsular, well demarcated, yellowish white, friable area was found. This was roughly pyramidal in shape and 25 mm. in diameter. In this area the liver markings were not distinct but there was no evidence of liquefaction or caseation necrosis. Further incisions disclosed two small similar areas in the substance of the liver. The small intrahepatic arteries often had narrow lumens and thick walls. The veins were normal. No thrombi were found in the branches or tributaries of the portal vein.

The gall-bladder and extrahepatic biliary ducts were normal.

Alimentary Tract: The esophagus was normal. Along the lesser curvature of the stomach, midway between the cardiac and pyloric orifices, there was a punched-out, sharply circumscribed, circular ulcer measuring 8 mm. in diameter. The flat firm base and indurated margins of the ulcer were covered by dark red, necrotic material. In the first portion of the duodenum, just distal to the pyloric sphincter and on the posterior wall, a large irregular ulcer which measured 20 by 10 mm. was found. This appeared to be of more recent formation than the gastric ulcer. The remainder of the duodenum and the proximal 10 cm. of the jejunum were normal. The distal portion of the jejunum had numerous, punctate, subserosal and submucosal hemorrhages. There was slight mucosal edema but no ulceration.

In the ileum three principal areas of hemorrhagic infarction with mucosal ulceration were disclosed. Other similar though less severe lesions were present. The first large infarct was in the first portion of the ileum. The second lesion was 25 cm. distal to the first (Fig. 2). The third major lesion was 35 cm. proximal to the ileocecal valve. They were from 2 to 5 cm. in length and involved the entire circumference of the ileum as well as the adjacent 5 to 10 mm. of the mesentery. Although the degree of hemorrhagic gangrenous necrosis was different in each instance, the lesions were of similar character. The wall of the intestine varied in thickness in propor-

tion to the extent of edema, hemorrhage and necrosis, but in several areas of each infarct it was thin, friable, and greenish black in color. The second lesion was the most severe. A hemorrhagic, fibrino-purulent exudate had accumulated on the peritoneum and the loop of intestine was bound to the diseased sigmoid colon by fibrous and fibrinous adhesions. The mucosa in each area of infarction was hemorrhagic, edematous, soft and ulcerous. The ulcerations for the most part were adjacent to the attachment of the mesentery. They were shallow, poorly demarcated and often confluent. Their bases were flat and were covered with necrotic, gray, hemorrhagic material. The regional mesentery was slightly edematous and congested but had been spared the extensive necrosis that characterized the ileum.

The colon, except for that portion between the middle of the descending colon and the rectosigmoid junction, was normal. The involved part of the colon was contracted. Its wall and mesentery were thick and indurated. The mucosa was hyperemic and extensively ulcerated. The ulcers varied from 8 to 15 mm. in diameter. They were disposed circumferentially and tended to lie between the edematous rugae. They had irregular, well defined margins and gray, firm bases. The thick, edematous and often densely fibrous submucosa formed their bases. The cicatrization of the edematous, pericolonic fat, and the firm, fibrous adhesions which bound the small bowel to the colon indicated that the process was of subacute or chronic character.

The rectum was normal.

The direct cause of the lesions of the ileum and colon was disclosed by a dissection of the superior and inferior mesenteric arteries. The main channels and all branches of greater than 2 mm. in thickness were normal. The arcuate arteries of about 1 to 1.5 mm. in thickness often showed definite thickening of their walls, moderate narrowing of lumens and an unusual degree of adherence of the adventitia to the regional fat of the mesentery. These vessels, however, were much less seriously involved than were their terminal branches which passed directly to the ileum. The degree of involvement was not uniform but varied from place to place along the intestine and from place to place in the same artery. The arteries which supplied the jejunum were relatively free from changes. Those of the upper ileum that were abnormal were few in number. Along the remainder

of the ileum the vascular lesions were rather uniformly distributed, although there was a tendency for groups of arcuate branches to be more seriously diseased than their neighbors. This was especially prominent in the vascular tree which supplied the areas of infarction (Figs. 3 and 4). The branches supplying the colon were only rarely affected. These small arteries, which were usually 1 mm. or less in diameter, were often pale gray and firm, resembling fibrous cords. In other instances this resemblance was restricted to local areas, while a more normal vascular tunic persisted between the lesions. Often the only portion of the vessel that showed prominent thickening was at the point where the artery entered the wall of the ileum. There were no definite aneurysms, although isolated, small, nodular thickenings and spherical, purplish intravascular masses occasionally were found. Cross-sections showed that the appearance of the vessels was due to an increase in the thickness of the adventitia which was often abnormally adherent to the adjacent fat tissue, to a fibrous thickening of the media and intima and to partial or complete closure of the lumens by dense white tissue or firm red thrombi.

The inferior mesenteric, left gastric and pancreaticoduodenal arteries were inspected. The smallest vessels presented lesions that were identical with those of the small arcuate branches of the superior mesenteric artery. These were prominent among the branches that supplied the sigmoid colon and the first part of the duodenum but were rarely found elsewhere.

Kidneys: The right kidney weighed 180 gm., and the left 160 gm. They were of similar appearance. The capsules, which were of uniform normal texture, were adherent to the cortex in a few, small, depressed areas. The external surface of each kidney cortex was irregular in contour. There were numerous broad, slightly concave depressions which surrounded and merged imperceptibly with slightly convex, plateau-like elevations. The elevations varied from 1 to 15 mm. in diameter. They were irregular in outline and were usually firm and purplish red in color. Rarely, they were soft and grayish yellow. The intervening areas of cortical substance were firm and grayish pink. Coronal sections of each kidney showed a continuation of these variations in color and texture deeply into the cortex with a gradual diminution in diameter as the medullary regions were approached. The kidney markings were well preserved in the pale gray areas, indistinct in the purplish red zones and ab-

sent in the homogeneous, friable, grayish yellow parts of the cortex. The pyramids, pelves and calices were normal (Fig. 1).

The main renal arteries and their primary branches were normal. The interlobular arteries showed a variable degree of mural thickening with concentric narrowing of their lumens. These changes became greater in the distal parts of these arteries and in the arcuate arteries. Here, the lumens of the vessels occasionally were reduced to minute channels or were replaced by dense fibrous tissue, so that the arteries resembled solid fibrous cords. Thus it seemed that the alterations in the renal cortex represented the effects of partial or complete deprivation of arterial blood.

Adrenals: The right adrenal weighed 15 gm., and the left 10 gm. The small extracapsular arteries had thick walls and narrow lumens.

Pelvic Organs: Normal in appearance.

Testes: Except for a firm indurated hemorrhagic area (3 to 4 mm.) beneath the tunica of one testis, the testes were normal.

Thyroid and Parathyroids: The thyroid and parathyroids were normal.

Bone Marrow: The marrow of the ribs was dark red in color and slightly diminished in amount.

Aorta: Rare atheromatous patches in the intima of the abdominal portion of the aorta were seen.

Eye: The vessels were engorged. Moderate edema of the retina was present. Pin-point hemorrhages were few in number.

Brain: The brain weighed 1340 gm. There was slight flattening of convolutions and narrowing of the sulci of the cerebral cortex. An indistinct pressure cone was present at the base of the cerebellum. The meningeal and cortical hemorrhages were restricted to the sites of operative trauma. Coronal sections disclosed numerous small, grayish brown areas (1 mm.) in the cerebral cortex and subjacent white matter. The arteries and their branches showed no lesions.

MICROSCOPIC STUDY

The sections were stained with eosin-methylene blue. The stains of Giemsa, Gram-Weigert and Levaditi were used in an attempt to demonstrate microorganisms.

Heart: The coronary arteries are normal. In the dense connective tissue adjacent to the adventitia of a small artery there is a

small area of infiltration with polymorphonuclear leukocytes and monocytes.

Lungs: All arteries are normal. An acute bronchitis and a confluent bronchopneumonia are present. Gram-positive diplococci are found in phagocytic cells. A few polymorphonuclear leukocytes are scattered throughout the walls of several veins.

Spleen: Except for a mild thickening and hyalinization of walls of arterioles, the arteries are normal. The sinusoids contain an increased number of polymorphonuclear leukocytes. A large amount of hemosiderin is found in mononuclear phagocytes. A hyaline, eosinophilic deposit is present in many follicles.

Stomach: There are lesions of the walls of submucosal arteries and ulcerations of the regional mucosa and submucosa. The lumens of the arteries are reduced to very narrow channels by proliferation of intimal cells and fibril formation. The newly formed intimal tissue is loose-textured and often infiltrated with polymorphonuclear leukocytes, plasma cells and lymphocytes. Occasionally, the intima is partially destroyed and thrombi fill the vascular lumens. In other arteries the process is less acute. In these instances cicatrization of the adventitia and media with lymphocytes scattered about in the dense connective tissue is found. The ulceration of the mucosa is acute. Edema, cellular infiltration and degeneration of tissues of the submucosa are noted in the base of the ulcer.

Duodenum: The ulcer is acute. There is extensive necrosis of the mucosa and submucosa. Thrombi of recent formation occlude many veins and arteries in the base and at the margins of the ulcer. The arteries of the submucosa, muscularis and serosa show pathological changes similar to those in the gastric arteries. The small arteries chiefly are affected by the disease. In the larger arteries intimal proliferation is predominant, and the vessel walls usually are free from inflammatory cell infiltration and fibrosis. The smaller arteries show less prominent intimal proliferation, old and recent thrombi, infiltration with lymphocytes and plasma cells and a variable degree of fibrosis of the media and adventitia.

Small Intestine and Mesentery: Numerous sections through the small intestine and its mesentery, both in the regions of the infarcts and of the grossly normal bowel, were studied. The significant findings are those in the walls of arteries and those of infarction of the ileum. The arterial lesions, which are common in the mesentery

and rare in the wall of the ileum, are inflammatory and chronic in character. The small arteries of less than 1 mm. in diameter principally are diseased. The smallest arteries, arterioles, and veins are normal or only secondarily affected. The greatest number of arterial lesions are healed. Several apparently are of chronic progressive character. None is acute. So far as could be determined, the earliest lesion seems to be characterized by an infiltration of polymorphonuclear leukocytes, plasma cells, lymphocytes and eosinophiles in the adventitia and outer portions of the media. Subsequent to, or coincident with, this reaction there is a proliferation of cells of the intima with deposition of delicate collagenous fibrils between the newly formed cells. This concentric proliferation is often so great that the narrowing of lumens is carried almost to the point of obliteration. In other instances degeneration of the inner layers of the intima and thrombosis has occurred. The various stages from acute thrombi to those which are dense, fibrous and canalized can be traced (Fig. 5). As the organization of thrombi progresses, there seems to be in the media a coincident atrophy and fibrosis accompanied by the appearance of small vascular channels. Cicatrization of the adventitia gradually becomes prominent. Numerous, small, endothelial-lined spaces are formed in the thick adventitia. These communicate through the newly formed capillaries in the media with the canaliculi in the thrombi. In several older lesions the cellular infiltration has disappeared, leaving the cicatrized remnants of the original arterial wall. There are several variations from this reconstructed sequence of pathological changes. The commonest variation is intimal proliferation which seems to be unassociated with inflammatory cell infiltration. The veins and large lymphatics show no primary lesions and only rarely are they seriously affected by the inflammation in the adventitia of the arteries which they accompany.

The wall of the ileum shows not only slight variations from normal histology but also extensive necrosis. In sections of normal ileum there is an occasional, small, submucosal artery which has a thickened intima and infiltrating lymphocytes and plasma cells in the adventitia. In the areas of necrosis, which may be considered as infarcts, arteritis is more widespread and severe. Not infrequently, acute and organized thrombi are found. In these regions there is an acute or subacute necrosis and a diffuse inflammation throughout the

entire wall. Usually the mucosa is destroyed. Beneath the ulcerations are many dilated vascular channels, several of which contain fibrinous thrombi. These thin-walled vessels extend through the acutely inflamed, edematous wall, ramify throughout the muscularis and serosa and anastomose with serosal vessels.

Colon and Mesentery: Numerous sections depict a process which is similar to that in the mesentery and wall of the ileum. The arteritis is of the same nature. The only appreciable difference between the infarcts is that in the colon there are several chronic, cicatrizing ulcers of the mucosa. In general, the lesions of the ileum are more acute.

Liver: In small arteries there are numerous inflammatory and proliferative lesions similar to those in the mesenteries, kidneys and elsewhere. The parenchyma is normal, except in the sections through the large pale area which is found along the anterior border of the liver. At the periphery of this well demarcated lesion small acute necroses are found in the midzonal regions. In the center of the area there is acute necrosis of the inner two-thirds of each lobule. The process is characterized by degeneration of liver cells, diminution in the number of red blood cells in the sinusoids, rare fibrinous thrombi in vascular channels and a large number of polymorphonuclear leukocytes, not only in sinusoidal spaces but also invading the columns of liver cells. This lesion is presumably an acute infarct which is the direct result of the arterial disease. In the periportal connective tissue and in Kupffer cells a moderate amount of hemosiderin is found.

Pancreas: There are numerous arterial lesions and old and recent infarcts. The type of arteritis is similar to that which has been described in the other organs (Figs. 6 and 7). Thrombi in all stages of organization are found. Several areas of acute or subacute necrosis and small intralobular cicatrices are present. These are probably secondary to partial or complete occlusion of the trophic arteries.

Kidneys: Arterial lesions and cortical infarcts are numerous. The inflammation of the arteries is either healed or of chronic progressive nature. The arteries which chiefly are affected measure less than 2 mm. in diameter. The interlobular and arcuate arteries show the most prominent changes. The smallest arteries, arterioles and glomeruli exhibit no primary lesions, although they are affected secondarily by closure of the vessels from which they arise.

The arterial disease is similar to that which is found especially in the mesenteries of the ileum and colon and in the pancreas. The largest arteries (2 mm.) show great thickening of the intima and a corresponding degree of reduction in the size of their lumens. This loose-textured, intimal hyperplasia is not associated with an inflammatory cell infiltration of the intima, except in those instances in which acute thrombi have formed. In the smaller arteries the fibrils and cells of the hyperplastic intima are in a more compact arrangement. The lumens are very narrow and often are occluded by old or recent thrombi. Recent thrombi are more common in the vessels of the kidney than elsewhere. However, the presence of organized and canalized thrombi, as well as the dense fibrosis of the media and adventitia, indicate that the disease is of long duration. In fact, there are only rare arteries which are infiltrated or surrounded by any significant number of lymphocytes and plasma cells.

The infarcts in the renal cortex are undoubtedly secondary to the arterial disease. As a rule, they are of acute nature and correspond to the elevated areas beneath the capsule of each kidney. Here, the glomeruli and tubules show a homogeneous necrosis without any appreciable disturbance of structural relations. The interstitial tissues are edematous and infiltrated with a few polymorphonuclear leukocytes. Infarcts of less recent date are represented by small radial cicatrices.

Adrenals: There are prominent pathological changes which involve the periadrenal tissues, the small arteries, the capsule, the sympathetic nerves and the cortex of each gland. The most significant lesions are those of the small arteries. These lesions consist of a proliferation of intimal cells in concentric fashion, an increase in the amount of collagen between the cells, marked narrowing of the lumens, organized and canalized thrombi, fibrosis of the adventitia and media and newly formed capillaries in the adventitia adjacent to the media. This process is accompanied by very little evidence of active inflammation. Occasional clusters of lymphocytes and plasma cells are found in the perivascular tissues and adventitia. The perivascular cicatrices which radiate from the adventitia involve the sheaths of several, unusually large, sympathetic nerve trunks. The fibrous capsule of each gland in many areas is dense and thick and portions of the glomerular zone are replaced by

cicatricial tissue. In the zona fasciculata there are acute and healed foci of degeneration. The acute lesions resemble the necroses in the liver. They show acidophilic, shrunken, cortical cells flanked by large numbers of polymorphonuclear leukocytes in the sinusoids. In the healed lesions most of the cortical cells have disappeared. Between the columns of residual atrophic cells there is an increased amount of connective tissue. In the medullary regions there are aggregates of sympathetic ganglion cells and large sympathetic nerve trunks.

Testis: Beneath the tunica albuginea there are several small arteries with lesions which do not differ from those in several other organs. These arteries contain several recent and a few organized thrombi. Focal areas of cicatrization and acute necrosis involve groups of seminiferous tubules. No significant changes are found in the arteries between the tubules.

Thyroid: The arteries and parenchyma are normal.

Pituitary: In the capsule of the anterior lobe there is an area of recent hemorrhage with fibroblastic proliferation. The arteries are normal.

Prostate and Urethra: At the periphery of the gland several small arteries show intimal thickening, which is due to a concentric deposition of dense lamellae of collagen. In the adventitia and less frequently in the media of these arteries there are groups of lymphocytes and plasma cells. A slight chronic inflammatory reaction is present in the mucosa of the urethra and the stroma of the prostate.

Aorta: The wall of the aorta is normal. In the adjacent fat tissue there are several small arteries with thickened intimal layers, narrow lumens and areas of lymphocytic infiltration in the adventitial connective tissue.

Abdominal Lymph Node: In the sinusoids numerous mononuclear phagocytes which contain red blood cells and hemosiderin are found. Eosinophiles are scattered through the gland. A small artery at the hilum shows concentric intimal hyperplasia, a narrow lumen and many small endothelial-lined channels in the thick fibrous adventitia.

Bone Marrow: The rib marrow contains an increased number of erythroblasts. A large amount of hemosiderin is demonstrable in large mononuclear phagocytes.

Voluntary Muscle and Skin: There are no lesions.

Retina: Between the outer and inner nuclear layers fibrinous thrombi occlude the lumens of several small capillaries. In two small areas necrosis and edema of the nerve fiber layer with hemorrhage into the retina and partial destruction of all layers internal to the pigment epithelium are found. The choroidal and retinal vessels are engorged, but no inflammatory lesions comparable to those in small arteries of other organs are detected.

Brain: Small areas of necrosis are numerous. These usually are characterized by hemorrhage and edema, some of which may be the result of operative trauma. However, several cortical and subcortical lesions show, in addition to hemorrhage and edema, a proliferation of glia cells, an infiltration with lymphocytes and monocytes and a proliferation of fibroblasts in the adventitia of small arteries. Acute thrombi are found in the lumens of occasional small vessels. A few lesions appear to be of several days duration. Other significant findings are occasional lymphocytes around small vessels in the medulla and pons and distinct small areas of myelin degeneration.

Special Stains: Numerous sections were stained by Wolbach's modification of the Giemsa stain and by the method of Gram-Weigert. These stains disclose no microorganisms that might be considered as etiological agents.

Sixty blocks of tissue from various organs were stained by Levaditi's method for the impregnation of spirochetes. In addition several blocks of cerebral tissue were treated by Jahnke's modification of the Levaditi and Manouélian method for the demonstration of *Treponema pallidum* in the brain and meninges. Only one section showed definite spirochetes although questionable forms appear in several sections. The spirochetes (Fig. 8) are located in the areas of necrosis and inflammation in the submucosa of the duodenum. They are morphologically consistent with *Treponema pallidum* and it seems justifiable to consider them as such.

Anatomical Diagnoses: Generalized syphilitic arteritis; multiple healed and acute arterial thrombi; acute and subacute infarcts of stomach, duodenum, ileum, colon, pancreas, liver, kidneys, adrenals, testes and brain; healed infarcts of pancreas, kidneys, adrenals and testes; localized acute and chronic peritonitis; bronchopneumonia; acute splenitis; chronic urethritis and prostatitis; fibrous perisplenitis and pleuritis; patent foramen ovale.

DISCUSSION

Instances of widespread inflammatory lesions of small arteries and arterioles have been observed and reported from time to time. Dickson² in 1908 reviewed the literature and added a case which he had studied. He discussed the lesions under two headings — Periarteritis Nodosa and Polyarteritis Acuta Nodosa. Opinion as to etiology has differed, but Dickson, along with others, believed that the former group was due to syphilitic infection and the latter group to some unknown infection or toxemia other than syphilis. Longcope³ the same year presented a case of periarteritis nodosa with an excellent clinical and pathological review of the disease. He pointed out the much greater frequency among males than females. Mott, who wrote the section "Arterial Degenerations and Diseases" in Allbutt and Rolleston's System of Medicine,⁴ agreed on the whole with Dickson as regards etiology and pathology. He discussed syphilitic disease of the small arteries and arterioles under the heading of Periarteritis Nodosa. This author emphasized the widespread distribution of the lesions, the frequent involvement of the coronary arteries and the early onset during the syphilitic infection. Allbutt⁵ is quite in agreement with Mott.

Warthin⁶ stated that syphilis involves the small arteries quite frequently. There is a large literature dealing with the effects of the syphilitic virus on the blood vessels, but practically all discussions are confined to observations on the cerebral and coronary vessels or the aorta and its larger branches.

When one consults modern text-books on pathology, such as those by Boyd⁷ and MacCallum⁸ little is to be found concerning the effects of syphilis on the small arteries and arterioles. Boyd does not believe that syphilis is the etiological agent in periarteritis nodosa. He uses as an argument the facts that periarteritis nodosa is chiefly a disease of the muscular type of arteries, while syphilis primarily attacks the elastic type.

Most authors agree that in syphilitic arteritis and in so-called periarteritis nodosa all coats of an artery may be involved. Opinions, however, differ as to the place of attack of the virus. The generally accepted explanation is that the lesion primarily occurs in the larger arteries about the vasa vasorum and in the smaller arteries in relation to the perivascular lymph channels.

The case presented here is of much interest from several points of view. The multiplicity, character and distribution of the symptoms, together with a hypertension of recent development, indicated that the patient had some disease of the vascular system. Longcope³ pointed out that these findings, together with a leukocytosis and fever, would suggest the diagnosis of periarteritis nodosa, even in the absence of superficial vascular nodules. The fact that the patient had been quite well until he contracted his syphilitic infection, which was of recent occurrence, made it seem probable that his illness was due to this infection. Mott⁴ and again Allbutt⁵ noted that the widespread involvement of the small arteries by the syphilitic virus occurred early in the course of the infection; usually during the secondary stage, although not infrequently during the late primary stage, and that the whole course of the illness was usually from a few weeks to months in duration. These observations were borne out in our case.

The gross examination of the organs of this patient yielded no clue which would allow diagnoses other than those of disseminated arteritis and multiple infarcts. The assumption at the time of the postmortem was that idiopathic periarteritis nodosa was the malady which would explain all findings in the most satisfactory manner. It was only after microscopic study that the diagnosis of syphilitic arteritis was made. The histological study disclosed inflammatory changes in the walls of small arteries and in the periarterial tissues. There were a few vessels which were affected by a relatively acute inflammation. Most of the lesions were chronic or healed. It was impossible to determine what part of the vascular wall was the original site of the infection. It seemed that the outer portion of the media and the adjacent adventitia were affected primarily. Fibrosis of the vessel walls was prominent. The partial or complete occlusion of the lumens of the arteries by concentric thickening of the intima and thrombosis resulted in the formation of numerous infarcts.

Inasmuch as periarteritis nodosa has not been established as an entity and because the term has come to be used as an opportune "label" for most instances of arteritis of unknown etiology, it seemed that certain features of the present case favored a diagnosis of syphilitic arteritis rather than periarteritis nodosa. In the first place, the patient had contracted syphilis a few months before the

onset of his fatal illness. Secondly, the appearance of severe signs and symptoms occurred during the period in which generalized syphilitic arteritis is most likely to occur. Thirdly, although the patient was treated promptly with arsphenamine and mercury, the therapy was interrupted for several weeks before the onset of serious symptoms. This lapse of therapy would allow for an intense exacerbation of the disease such as, according to Moore,⁹ not infrequently occurs in the central nervous system in instances of primary or early secondary syphilis under similar circumstances. Fourthly, the histology was more compatible with syphilis than any other disease. Fifthly, spirochetes, which were indistinguishable from the *Treponema pallidum*, were found in an area of necrosis in the submucosa of the duodenum.

Generalized syphilitic involvement of the small arteries must be uncommon. This is the first case of this type recognized in this hospital. It is impossible to state why the vascular system should have been so vulnerable to the virus. Stokes¹⁰ states that both arsphenamine and mercury are toxic to the walls of blood vessels. In this case treatment was instituted early and was intensive over a period of several months. It may be that this is an example of the "therapeutic paradox" following the use of arsphenamine. Stokes¹⁰ believes that this is due to the too rapid healing of the lesions. He states that "closure of a partially obliterated coronary vessel or branch by edema at some spot of active gummatous arteritis, as in the Herxheimer flare-up, or rapid strangulation by too quick scar formation and healing, may produce death before any compensatory mechanism can come into play." It is conceivable that some such occurrence took place in the present case. Another explanation which has been offered, especially in instances of neurorecurrences of which this is an example, is that early intensive treatment may have prevented the natural development of adequate bodily defences against the virus and that the lapse of treatment permitted a severe exacerbation of the disease.

SUMMARY AND CONCLUSIONS

☞ A case of widespread progressive chronic arteritis in a young adult with syphilis has been described. The small arteries were involved almost exclusively. All three coats of the vessels were in-

cluded frequently in the lesion. In most instances the vessels were occluded partially or completely, either by the marked thickening of their walls, by the formation of thrombi, or by a combination of the two processes. Infarcts of various organs often were present in regions where the most severe vascular lesions were found. The etiology of the arteritis has not been proved conclusively, but for several reasons cited in the discussion we believe it to be the virus of syphilis.

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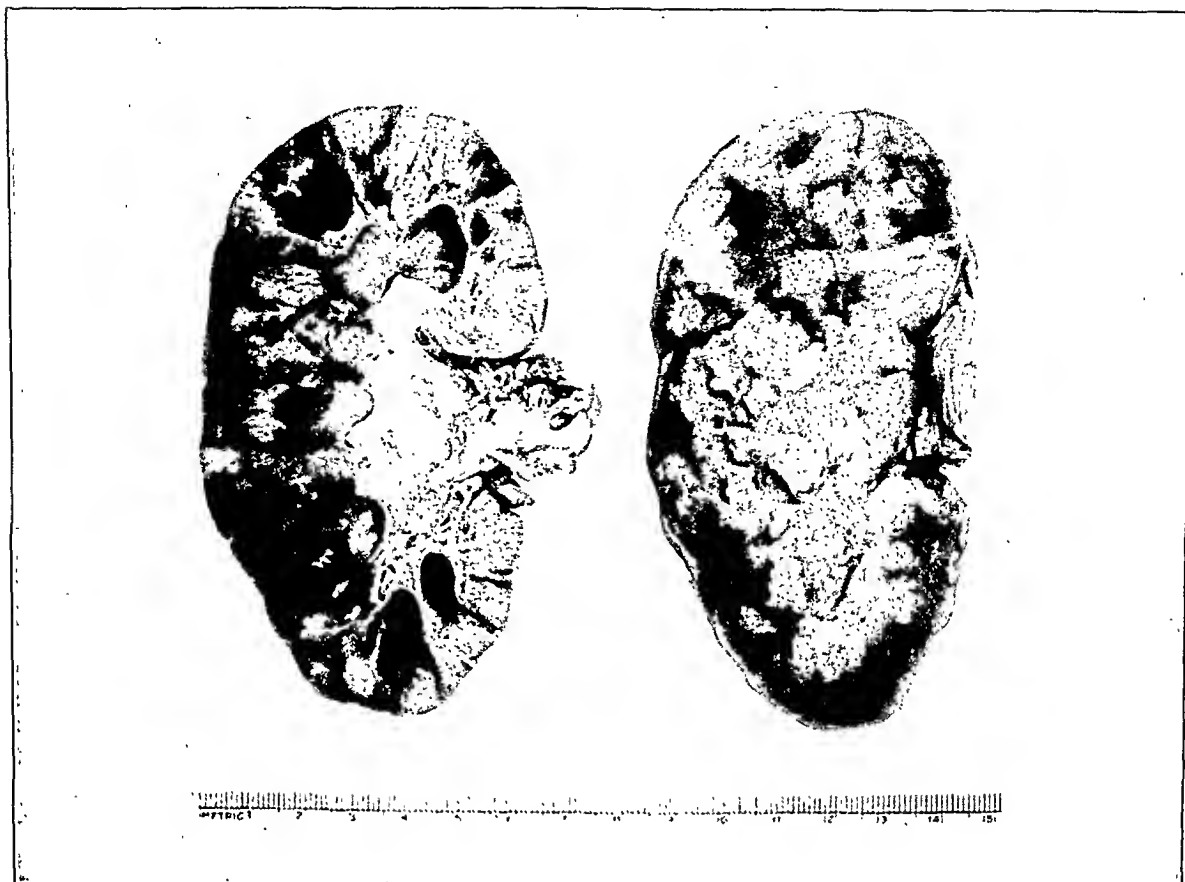
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DESCRIPTION OF PLATES

PLATE 44

FIG. 1. Photograph of a coronal section of the kidney and of the external surface from which the capsule has been removed.

FIG. 2. Photograph of a distended segment of the ileum. Note the two areas of infarction.



I



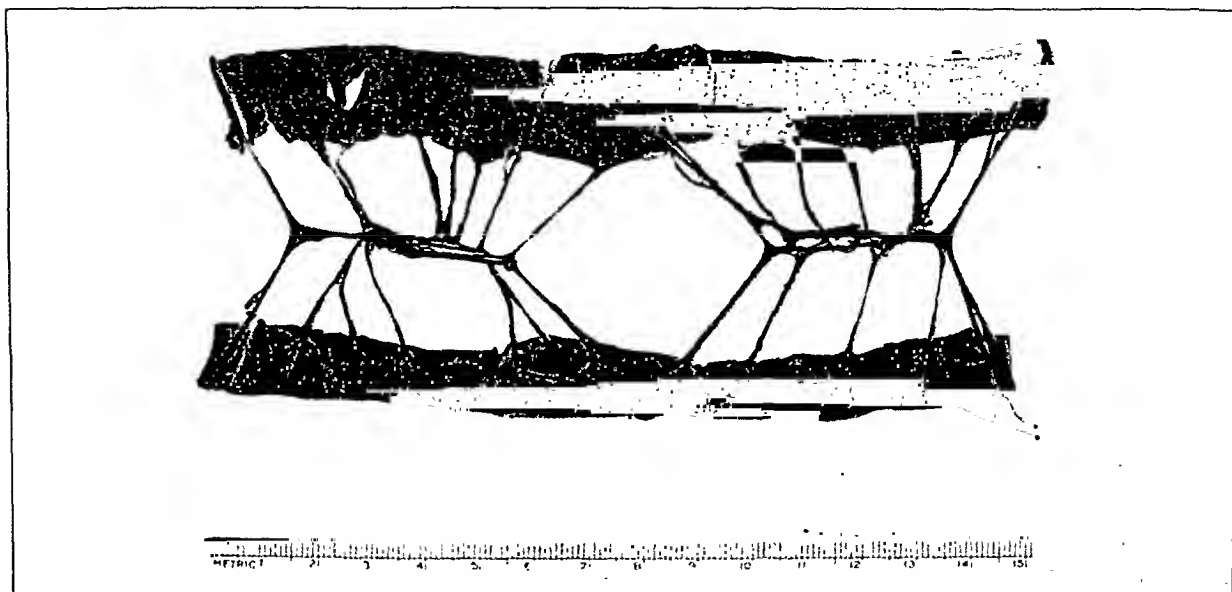
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PLATE 45

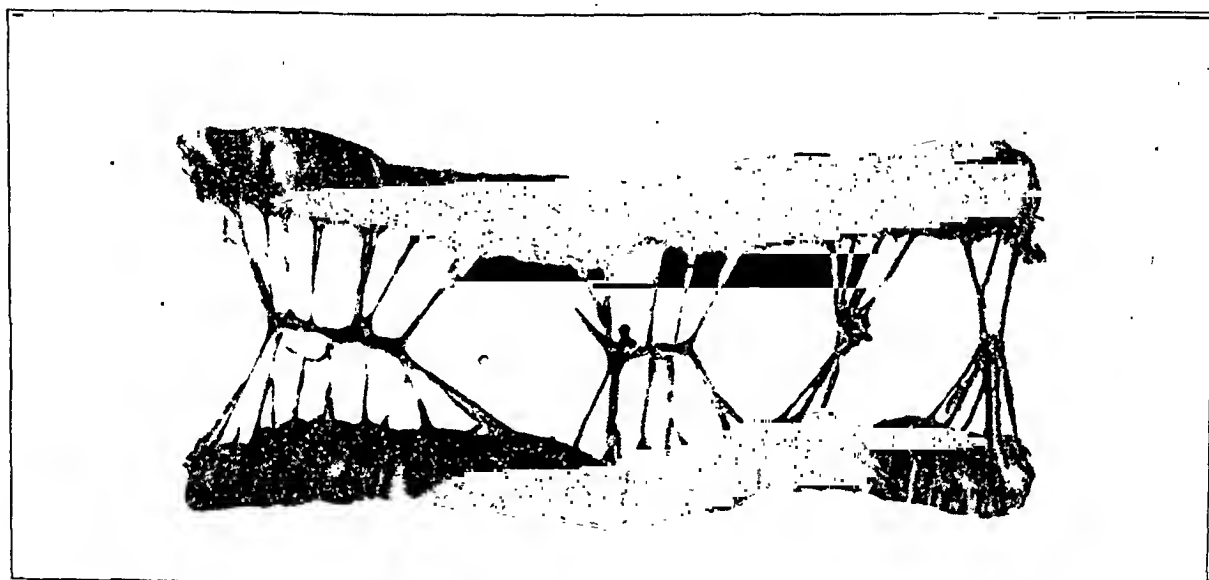
FIG. 3. The specimen for this photograph was prepared in the following manner. A segment of a normal ileum was selected. A dissection of the terminal arcuate branches of the superior mesenteric artery was made. The ileum was then bisected in the plane of the mesentery and the divided halves of the wall spread out so as to show the paired arcuate branches in one plane.

FIG. 4. The specimen for this photograph was selected from a portion of the ileum between the areas of infarction in the present case. It was prepared in the same manner as the normal ileum in Fig. 3. Note the increased opacity and thickness of parts of the arcuate arteries. This illustrates the scattered distribution of prominent lesions without true modosities or aneurysmal dilatations. The vessels from which these arteries arose were normal.

FIG. 5. Photomicrograph of a thickened terminal arcuate artery (such as those in Fig. 4) and an accompanying vein. Note the fibrosis, vascularization and cellular infiltration in the media and adventitia. This also exemplifies the typical intimal proliferation with consequent narrowing of the vascular channel. $\times 100$.



3



4



5

PLATE 46

FIG. 6. Photomicrograph of the pancreas. Note the extraordinary intimal proliferation in the large artery. The two small arteries are similar histologically to the arcuate artery in Fig. 5. A small area of parenchymal fibrosis is also included in the picture. $\times 25$.

FIG. 7. Photomicrograph showing the type of lesion found in the large artery in Fig. 6. It illustrates the typical pathology of the largest vessels which were involved by the disease. $\times 100$.

FIG. 8. Photomicrograph of one of the *Treponema pallidum* in the wall of the duodenum. $\times 2000$.



6



7



8

CONGENITAL ANOMALY OF THE HEART *

REPORT OF A CASE; WITH EMBRYOLOGICAL DISCUSSION

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As far as the author is aware, such a combination of congenital anomalies of the heart as persistence and detorsion of the bulbus cordis, partial transposition of the aorta, interauricular and interventricular septal defects, patent ductus arteriosus, sinistroposition of the right auricle and right aortic arch has hitherto rarely been recorded in the medical literature. One of the cases (Case 9) reported by Wenner¹ seems to correspond fairly closely to such a description. This author, however, labelled it *cor monoventriculare biatriatum*, in spite of the presence of a low but thick interventricular septum on the anterior ventricular wall about two finger breadths above the apex. A case with this combination of anomalies, which was under observation for about 7 weeks in the pediatric service of the Peiping Union Medical College Hospital, presents a few anatomical features that seem to shed a new light on some of the aspects of our knowledge of the embryology of the heart. For these reasons a detailed report of the case with a discussion of the mechanism of its development seems warranted.

REPORT OF CASE

Clinical History: S. H. M., a Chinese female infant, aged 53 days, was admitted to the pediatric service of the Peiping Union Medical College Hospital on Dec. 20, 1929, for frequent cough of more than 20 days duration. After birth the patient had been apparently normal, except for some edema of the legs, which practically disappeared in 10 days. Fifteen or 16 days after birth she began to have some nasal discharge and frequent cough. She cried often and had attacks of cyanosis, rapid and difficult respiration, perspiration, occasional vomiting and, later, unconsciousness. There had been no other complaints. She was born at term and spontaneously, and never had any illness, except as mentioned. The parents were healthy and denied venereal disease. The mother had had one other pregnancy, a girl 4 years of age, living and well.

On admission the important physical findings were as follows: temperature 37° C.; pulse rate 168 per minute; respiration rate 42 per minute, at times

* Received for publication August 25, 1934.

more rapid and labored; dark color of the skin; moderate cyanosis of the mucous membranes; slight enlargement of the cardiac dullness toward the left; aortic second sound slightly accentuated; a fairly loud, short, blowing systolic murmur over the whole precordium; slight dullness with diminished breath sounds over the anterior right upper region of the chest; medium moist râles over lower posterior portion of the chest on both sides, and definitely palpable liver edge 1.5 cm. below the costal margin.

After admission the patient was found to be excitable and had cyanosis and dyspnea during convulsions, which could be curtailed by the administration of chloroform or morphine. Typical "obstetrical hand" was noticed on one occasion only. Roentgenological examinations of the chest done on Dec. 21 and 31, 1929, and Jan. 13, 1930, revealed persistent opacity in the region of the right upper lobe of the lung, and the possibility of an azygos lobe was suggested. On January 8th an abscess of the left thigh was incised. On January 19th a furuncle of the right external auditory canal was noticed and treated.

During the patient's stay in the hospital her condition and physical findings, except as noted above, remained stationary. On February 5th she had another attack of cyanosis and dyspnea, for which morphine injection was given. She failed to recover and died, presumably of respiratory failure, after having lived 100 days.

The laboratory findings were as follows.

Urine: Acid in reaction, 1 plus albumin, occasionally positive sugar reaction, a few white blood cells and granular casts in sediment.

Blood: On admission red blood cell count 5.03 million per cmm., with 134 per cent hemoglobin value (Sahli). White blood cell count 6800 per cmm. with 53 per cent polymorphonuclear neutrophils, 1 per cent eosinophiles, 45 per cent lymphocytes and 1 per cent monocytes.

On Feb. 2, 1930, the red blood cell count was 4.2 million per cmm., with 89 per cent hemoglobin value (Sahli).

Feces: No protozoa or ova.

Clinical Diagnoses: Congenital malformation of the heart; questionable patent ductus arteriosus; bronchopneumonia, left lower lobe, microorganism undetermined; atelectasis, right upper lung; otitis externa.

AUTOPSY REPORT

The autopsy was done approximately 21 hours after death. The body was that of a well developed but poorly nourished, Chinese female infant weighing 3 Kg. and measuring 54 cm. in length. Rigor mortis was moderate and livor mortis was present over the dependent portions. The skin was dark colored, the mucous membranes definitely purplish. The skull was of normal contour; the anterior fontanelle was open, 1.5 by 1.2 cm., the posterior fontanelle closed. A small amount of thin yellowish discharge was present in the right auditory canal. Eyes and nose were normal. The abdo-

men was slightly distended. Extremities showed no clubbing of fingers or toes and no other abnormal findings. The external genitalia were normal.

The peritoneal cavity contained a small amount of clear yellowish fluid; the surfaces were smooth and glistening. The omentum was free in the cavity. The intestines were moderately distended with gas. The liver and spleen were in their normal positions.

The right and left pleural cavities contained no excess of fluid and their surfaces were smooth and glistening. The lungs were voluminous. The pericardial sac contained a few cc. of clear yellowish fluid. The heart was much enlarged and, as its exposure revealed an apparent malformation, the body was immediately embalmed so as to allow a careful dissection of all the larger blood vessels. The thoracic and abdominal organs were removed *in toto*.

The heart was much enlarged and was approximately three times the normal size of that of a 3 months old infant. Its shape was like that of a pear, with the anteroposterior diameter longer than the transverse, being 4 and 3.5 cm. respectively. The greatest circumference around the ventricles was 12.4 cm. and the vertical length from the level of the origin of the aorta to the inferior border was 5.2 cm. From the sternocostal surface (Fig. 1), the most striking abnormality was the position of the aorta. It arose from the extreme right upper conus of the heart directly anterior to the origin of the pulmonary arterial trunk. On the left side there lay both the right and the left auricular appendages, the former being the more cephalic of the two. The apex was globular and formed entirely by the left ventricle. The diaphragmatic surface (Fig. 2) showed that a greater part of the upper portion was occupied by the left and right auricles, into which their respective veins entered, and a smaller part was occupied by the arterial trunks at the extreme upper right portion. The lower portion of this surface is the ventricular area. Between the sternocostal and the diaphragmatic surfaces was the acute margin (Fig. 3), which was by no means distinct and on which the marginal branch of the circumflex artery ran. The left surface of the heart, or the obtuse margin, was broad and showed the origin of the aorta from the ventral and cephalic ventricular portion, the pulmonary arterial trunk being hidden. The right and left auricular appendages were clearly shown to lie in apposition to each other.

The aorta, after its origin from the right ventricle, where it measured 1.3 cm. in diameter, ran cephalad and dorsad, and was ventral and to the left of the pulmonary arterial trunk. It gave off the left innominate, the right carotid and the right subclavian arteries in the order named, about 2.5 cm. from the level of its origin. The aorta communicated with the right branch of the pulmonary artery by a patent ductus arteriosus, which measured 4 mm. in length and barely admitted a probe, so that the diameter of its lumen was not more than 1.5 mm. At this level the aorta arched over the right bronchus and was continuous with the thoracic and abdominal portions of the aorta, which were normal.

The pulmonary arterial trunk also took its origin directly dorsal to the aortic orifice, and measured about 1.4 cm. in diameter at this level. It closely followed the course of the aortic arch and remained dorsally and on the right. At the level where the left innominate artery arose from the aorta, the pulmonary arterial trunk branched. The right branch, after giving off the ductus arteriosus, ran dorsad and toward the right to the hilum of the right lung, while the left passed caudad to the transverse aorta and reached the hilum of the left lung. Both of these arteries were moderately dilated.

Because of the malformation, it was not practicable to open the heart by the usual technique of following the blood stream. Thus, the pulmonary veins and the left auricle were opened first. It was noted that the auricle was apparently normal, being lined by smooth and glistening endothelium with its usual pectinate muscles in the appendage. The interauricular septum was found to be normal in appearance, but the foramen ovale was patent, measuring 8 by 4 mm. There was no trace of the foramen primum. When one looked into the left ventricle the mitral orifice appeared to be irregularly oval in shape and was 11 and 7 mm. in diameter. The ventricular wall was then cut between the two normal papillary muscles, to which were attached by normal chordae tendineae a large anterior and a small posterior cusp of the mitral valve. As the left ventricle was thus exposed, the interventricular septum appeared normal (Fig. 4), but when the anterior leaflet of the mitral valve was elevated a septal defect measuring 9 by 5 mm. was present at its cephalic end. It was roughly U-shaped with a smooth and rounded edge. The height of the left ventricular surface of the interventricu-

lar septum measured 19 mm. The left ventricle communicated with the left auricle through the mitral orifice and with the right ventricle through the septal defect. No efferent vessel was derived from it. The left ventricular wall measured 11 mm. in thickness.

The right auricle was then exposed, showing no abnormal findings other than the defect in the interauricular septum above described. The tricuspid orifice was sickle-shaped, measuring about 15 mm. in length. An oblique cut was then made on the right ventricular wall. The arterial trunks were cut open longitudinally and the incisions extended to the right ventricular wall until they met. In such a way, a V-shaped portion of the ventricular wall could be lifted up, exposing the right ventricular cavity (Fig. 5). This was found to be smaller than the left ventricle and was cephalad, ventral and to the right of it. The thickness of the right ventricular wall measured 9 mm. The tricuspid cusps were normal and were provided with normal chordae tendineae and three short but plump, papillary muscles.

The right ventricle communicated with the right auricle through the tricuspid orifice, with the left ventricle through the interventricular septal defect, and with the aorta and the pulmonary arterial trunk through their respective orifices. It was bounded on all sides by a rather thick muscular wall, the shape of which resembled the bulbus cordis in the heart of an early embryo. Caudad, it was separated from the left ventricle by the defective interventricular septum. Between the aortic and the pulmonary orifices and extending into the cavity there was a thin fleshy flap, the bulbar septum (Fig. 5), which measured from 7 to 11 mm. in height.

The aortic orifice was guarded by three normal appearing semilunar cusps (Fig. 8, *b*), one anterior, one right posterior and one left posterior. From the right posterior sinus of Valsalva there arose the right coronary artery. A shallow dimpling was present on the aortic wall in the left posterior sinus of Valsalva, representing the rudimentary left coronary artery.

The orifice of the pulmonary arterial trunk was also guarded by three normal semilunar cusps, one posterior, one right anterior and one left anterior. The aperture of this trunk was slightly larger than that of the aorta.

After suturing some of the cuts above mentioned, the heart was then divided into two halves by extending the cut in the left ventric-

ular wall so that each half carried one arterial trunk (Fig. 6). In such a way a remarkable picture was produced. It was noted that the right ventricle, from which the two arterial trunks arose, was composed of a conically shaped muscular tube corresponding in shape and position to the bulbus cordis of an early embryonic heart. The orifices of the arterial trunks were at a much higher level than the auriculoventricular orifices. The interventricular septum took its origin from the ventral surface at a level between the middle and lower thirds. It was placed in such a plane that the right ventricular surface pointed cephalad, slightly ventral and to the right, while the left ventricular surface faced caudad, slightly dorsal and to the left. Its free margin was directed toward the caudal end of the interauricular septum, but was separated from it by the interventricular foramen. The left ventricle was thus at a lower plane than, or caudad to, the right. The tricuspid and mitral orifices were approximately at right angles to the interventricular septum.

The right coronary artery (Fig. 7), after taking its origin from the right posterior aspect of the aorta, emerged between the two arterial trunks and ran laterally to the right for a short distance. Then it branched into two, one circumflex and one infundibular branch. The former traveled posteriorly and toward the left in the coronary sulcus and gave off (1) a marginal branch at the level of the right border of the pulmonary trunk running along the acute margin; (2) a posterior descending ramus, which ran downward over the posterior surface, its branches terminating over the lower border of the sternocostal surface; (3) a number of posterior rami on the left ventricle supplying the left ventricular wall; and (4) a terminal branch which ended over the left surface of the heart. The infundibular branch ran obliquely anteriorly and soon gave off (1) an anterior ramus, which ran obliquely downward and slightly to the left at the right border of the heart, terminating at about the lower fourth of the anterior surface; and (2) an anterior descending ramus, which wound transversely around the infundibulum of the aortic trunk and reached the border of the right auricular appendage, where it turned sharply so that it ran obliquely downward and slightly toward the right, terminating at a point 1.8 cm. from both the right and the inferior borders of the heart on the anterior surface.

The right lung was three lobed, but an extra fissure was present over the lower lobe and over the posterior surface of the upper lobe.

The latter corresponded to the sulcus usually formed when an azygos lobe of the lung exists. Although at time of autopsy the presence of an azygos vein was unfortunately not definitely ascertained, the clinical and pathological pictures fit in well with such a condition. The left lung consisted of one lobe; however, a few shallow fissures were present over the surface. The pleural surface was smooth and glistening. The consistence was soft and crepitant, except in the right upper region where it was slightly firm. On section the cut surface showed atelectasis of the azygos lobe. The bronchi and the bronchial lymph nodes were normal.

The other organs showed no noteworthy findings.

As a whole, the microscopic study of different organs was not remarkable. The section of the heart showed normal myocardium. The sections of the lungs showed a moderate degree of emphysema in all the lobes, except the right upper lobe where a partial atelectasis and some leukocytic exudate in a few alveoli were present.

Anatomical Diagnoses: Congenital anomaly of the heart with persistence and detorsion of bulbus cordis, partial transposition of the aorta, interauricular and interventricular septal defects, patent ductus arteriosus, sinistroposition of the right auricle and right aortic arch; hypertrophy of the heart; anomaly of the coronary arteries; partial atelectasis and lobular pneumonia of azygos lobe of the lung; anomaly of the lung (single lobe of left lung with fissures, small fissures in the right lower lobe).

DISCUSSION

In his study on the development of the human heart from its first appearance to the stage found in embryos with twenty paired somites (1.5 to 3.3 mm.), Davis² reports that the embryonic heart is composed of aortic-bulbar, cardiac-bulbar, ventricular and atrial regions, demarcated by the interbulbar, bulboventricular and atrio-ventricular sulci ectally, and their corresponding ridges entally. With the growth of the cardiac tube much exceeding that of the pleuropericardial sac, and with the difference in the rate of growth in different regions of the heart, there occurs a bending of the cardiac tube, forming an S-shaped structure and a shifting in the positions and a change in the relative exaggeration or obliteration of the different sulci and ridges. In all of the embryos that he studied, these landmarks are definitely present.

In almost all the standard text-books of human embryology it is noted that in the embryo of about 5 mm. a definite sulcus appears at the bottom of the common ventricle, marking out the future right and left ventricles, and another slightly less definite one on the right side between the bulbus and the right ventricle. In the embryo of about 10 mm. the rudimentary interventricular septum appears between the dilated right and left ventricles, with a corresponding ectal interventricular sulcus. Another shallow ectal sulcus (the right bulboventricular) and a corresponding low ental ridge or spur persist between the bulbus and the right ventricle. In the embryo of about 15 mm. the interventricular septum is set vertically on the floor of the common ventricle and directed toward the auriculoventricular endocardial cushions, thus incompletely dividing the common ventricle into two chambers. With further development of the right ventricle, the right bulboventricular ridge becomes obliterated.

The bulbus cordis is only a transient, but important, structure in the development of the human heart. It soon becomes incorporated with the right and the left ventricles, mostly the former, and forms the infundibular portions.

In the above review there are a few points that seem to be rather confusing. The value of definite landmarks in the interpretation of morphological development has been demonstrated in the works of Schulte,³ Murray⁴ and Yoshinaga⁵ in the development of the mammalian heart. It is definitely shown by Davis² that there exists an interbulbar sulcus and ridge in the heart of embryos at, or before, the stage of twenty paired somites. This landmark is neither represented nor mentioned in the studies of the hearts of embryos of 5 mm. or more. What becomes, then, of this sulcus and ridge in the heart of these embryos? The interventricular sulcus, which denotes the origin of the interventricular septum, appears in the hearts of embryos of 5 mm. or more, but where is this sulcus in the hearts of embryos at, or before, the stage of twenty paired somites? Unless one assumes that the interventricular sulcus does not appear in the hearts of embryos before, and the interbulbar sulcus becomes entirely obliterated in the hearts of those after, the twenty paired somites stage, both of which are not likely to be the case, this discrepancy is not conceivable.

In the case reported here it is noted that the right ventricle con-

sists of a thick, muscular, tubular structure corresponding in position and shape to the bulbus cordis of an early embryonic heart. The interventricular septum arises from the ventral wall at a level corresponding to that of the bulboventricular ridge in the hearts of embryos of from 2 to 3 mm., runs obliquely and slightly cephalad, and fuses with the auriculoventricular endocardial cushions. It is also noted that the orifices of the arterial trunks are at a much higher level than the auriculoventricular ones, corresponding to the situation existing in a heart of an early embryo. Such a remarkable picture cannot be rationally explained by our text-book knowledge of the development of the heart, unless one assumes that the interventricular septum is malposed.

To clarify the confusing points, as noted above, one should scrutinize closely the difference in the interpretations of these observations. From his beautiful preparations, Davis² is justified in dividing the bulbus cordis into the aortic and cardiac portions, which are demarcated by the interbulbar sulcus ectally. The bulboventricular sulcus is a definite and important landmark, as will be discussed presently. On account of the simultaneous growth of the cardiac bulbus and the ventricular loop, which produces a downward displacement of the cardiac bulbus, the right bulboventricular sulcus shifts and appears at the bottom of the so-called "common ventricle" and is called by the older writers the interventricular sulcus. At the same time, the descent of the bulbus brings down the interbulbar sulcus, which has been erroneously considered as the bulboventricular sulcus. It seems reasonable, therefore, to consider that the bulboventricular sulcus of Davis and the interventricular sulcus are one and the same, and the interbulbar sulcus and the bulboventricular sulcus described in the text-books are also one and the same landmark. The interventricular septum is, therefore, developed from the right bulboventricular ridge of Davis.* With this change in the interpretations of these landmarks, it will follow that the ventricular loop of the early embryonic heart is destined to form the left ventricle, the cardiac bulbus the right ventricle of the adult heart, and that the aortic bulbus, by virtue of its torsion, the infundibular portions of both the right and the left ventricles.

* In this connection, Murray⁴ claims that the left bulboventricular ridge contributes to the formation of the interventricular septum of the adult heart of the rabbit.

Keeping this in mind, the findings in our case can be easily explained by an inhibition in growth and a lack of downward displacement of the cardiac bulbus, so that the interventricular septum remains in a position corresponding to that of the bulboventricular ridge of an early embryonic heart and the orifices of the arterial trunks maintain a higher level than the auriculoventricular orifices. Similarly, the case of *cor biatriatum triloculare*, reported by Holmes⁶ and republished by Abbott,⁷ in which a defective septum appears between the so-called "common ventricle" and a small cavity from which the pulmonary artery is derived, can be explained by the assumption that this septum represents the normal interventricular one, arises from the bulboventricular ridge, but appears to be "malposed" on account of an atrophy of the cardiac bulbus, and a hypertrophy and marked dilatation of the left ventricle.

In explaining most of the anomalies of the bulbus cordis it is a distinct advantage to separate the aortic and the cardiac portions of the bulbus, as a disturbance in one may not necessarily affect the normal development of the other.

Because of the lack of descent of the aortic bulbus in our case, the partial transposition of the aorta, *i.e.* both arterial trunks arising from the right ventricle, is naturally to be expected. In view of the recent work on the torsions of the bulbus cordis (*i.e.* the aortic bulbus) the anterior or ventral position of the aorta in relation to the pulmonary trunk can readily be explained on the detorsion basis. In order to get to the normal heart, the distal end of the aortic bulbus will have to make a clockwise rotation, or torsion, of about 180° . In the present case, as illustrated in Figure 8, the torsion stops at approximately 60° , hence the detorsion defect.

The final closure of the interventricular septal defect is normally made by the bulbar septum, which forms the membranous portion. This septum in the present case is well formed, but on account of the lack of descent of the cardiac bulbus and of complete torsion of the aortic bulbus, it is unable to close the interventricular foramen.

The sinistroposition of the right auricle closely resembles the case of Birmingham⁸ and that of Wenner.¹ The latter author has demonstrated clearly in his paper that this is due to the malposition of the aortic bulbus ("truncus arteriosus," according to this author). Normally, the aortic bulbus should ascend upward and toward the

left, passing in between and ventral to the right and left atrial pouches, but in these abnormal cases it passes directly upward on the right side of the right atrial pouch. It is conceivable that this malposition of the right auricle may have some inhibitory effect on the normal development of the right ventricle.

The right aortic arch is well discussed by many authors and it is sufficient here to explain it by the persistence of the right instead of the left fourth arterial arch. The left fourth arch instead of the right becomes the left innominate artery, and the persistent right sixth arch instead of the left the ductus arteriosus.

Anomalies of the coronary arteries are not rare. In the case here reported, the right coronary artery took its origin from the right posterior sinus of Valsalva, which usually gives rise to the left branch, but on account of the incomplete torsion of the aortic bulbus the left coronary artery became the right one. Because of the arrest in growth and absence of the other coronary artery the infundibular branch of the right one became compensatorily dilated.

Clinically there are remarkably few physical signs in this case, except a definite, loud, short, blowing systolic murmur heard over the precordium, and cyanosis and dyspnea during convulsions.

The intracardiac circulation of blood in this case (Fig. 9) undoubtedly involves a free mixing of the arterial and venous blood before it is sent out into the systemic bed. In a resting state of the patient cyanosis does not occur, probably because of the insufficient oxygen-unsaturation of the peripheral blood, or of the high "threshold value" in this particular case on account of its dark coloration of the skin. However, when the oxygen-unsaturation is raised above this "threshold value," as during convulsions, cyanosis occurs. The finding of partial atelectasis and pneumonia in the azygos lobe of the lung may contribute in a slight degree to the production of cyanosis in this case. Thus, in the terms of Lundsgaard and Van Slyke,⁹ the cyanosis in our patient is mainly produced by the *a* and *D* factors, and slightly by the *l*-factor.

SUMMARY AND CONCLUSIONS

An unusually rare case of congenital anomaly of the heart with persistence and detorsion of the bulbus cordis, partial transposition of the aorta, septal defects, patent ductus arteriosus, sinistroposi-

tion of the right auricle and right aortic arch is described in detail. Anomalies of the coronary arteries and of the lung are also noted.

It is suggested that the interventricular septum develops from the bulboventricular ridge of the early embryonic heart. With the arrest of growth of the cardiac bulbus resulting in a lack of downward displacement of the right ventricle, which is developed from the cardiac bulbus, and a lack of descent and detorsion of the aortic bulbus, the almost horizontal position of the interventricular septum, the high level of the orifices of the arterial trunks and the conical shape of the right ventricles are explained.

Clinically, there are remarkably few physical signs, so that an exact ante mortem recognition of this condition is almost impossible. Cyanosis occurs only during physical exertion.

NOTE: The author wishes to acknowledge his indebtedness to Dr. I. C. Wen, of the Department of Anatomy, and Dr. C. E. Forkner, of the Department of Medicine, Peiping Union Medical College, Peiping, China, for valuable criticisms in the preparation of this paper.

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KEY TO THE PLATES

A O = Aorta	P A = Pulmonary artery
B S = Bulbar septum	P V = Pulmonary vein
D A = Ductus arteriosus	R A = Right auricle
D I = Diaphragmatic surface	R C = Right carotid artery
F O = Foramen ovale	R S = Right subclavian artery
I C = Inferior vena cava	R V = Right ventricle
L A = Left auricle	S I = Septum primum
L I = Left innominate artery	S II = Septum secundum
L P = Left pulmonary artery	S C = Superior vena cava
L V = Left ventricle	S C = Sternocostal surface (in Fig. 7 only)
M A = Acute margin	T O = Tricuspid orifice
M O = Obtuse margin	T V = Tricuspid valve
M V = Mitral valve	V S = Interventricular septum

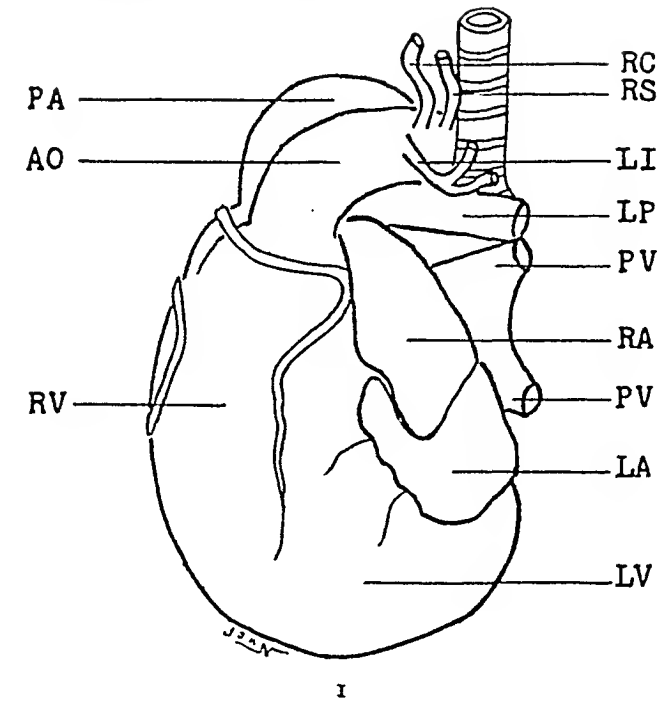
DESCRIPTION OF PLATES

PLATE 47

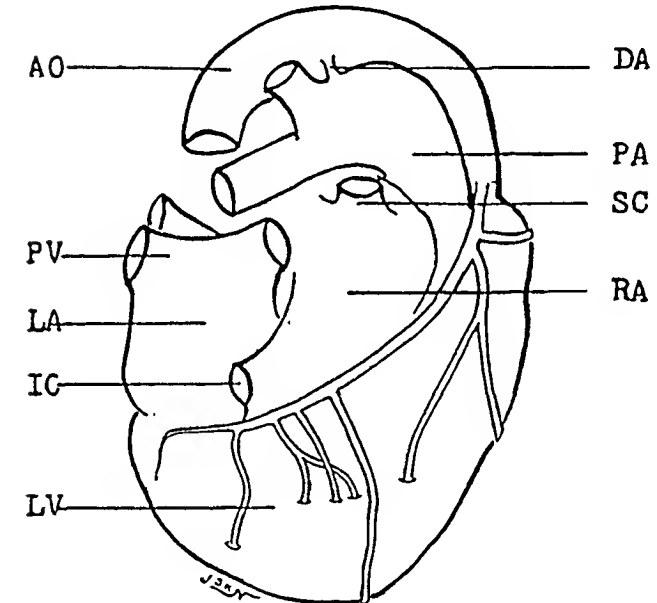
FIG. 1. Sternocostal surface showing origin, position and course of aorta in relation to pulmonary artery and trachea, the sinistroposition of the right auricle and the general shape of the heart.

FIG. 2. Diaphragmatic surface.

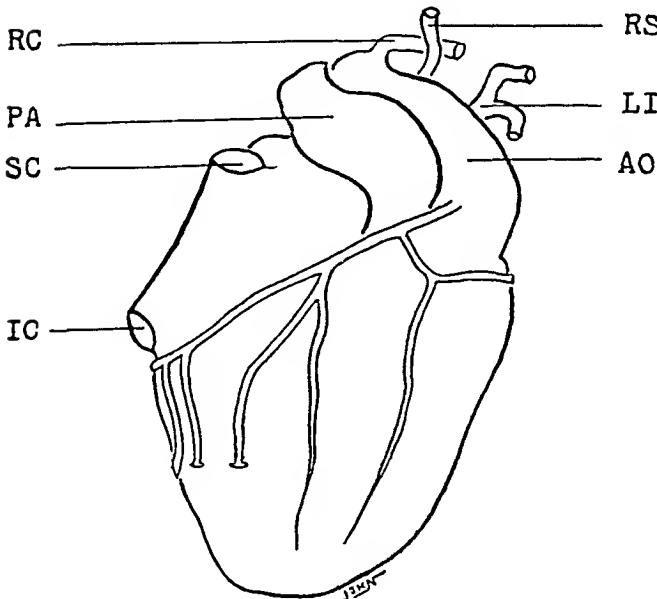
FIG. 3. Acute margin.



I



2



3

PLATE 48

FIG. 4. Showing interior of left auricle and left ventricle.

FIG. 5. Showing the left and right ventricle, the interventricular septum with its defect and the origin of the arterial trunks from the right ventricle.

FIG. 6. Showing the interior of the heart.

1 = Pulmonary artery

2 = Aorta

3 = Right ventricle

4 = Left ventricle

5 = Right auricle

6 = Left auricle

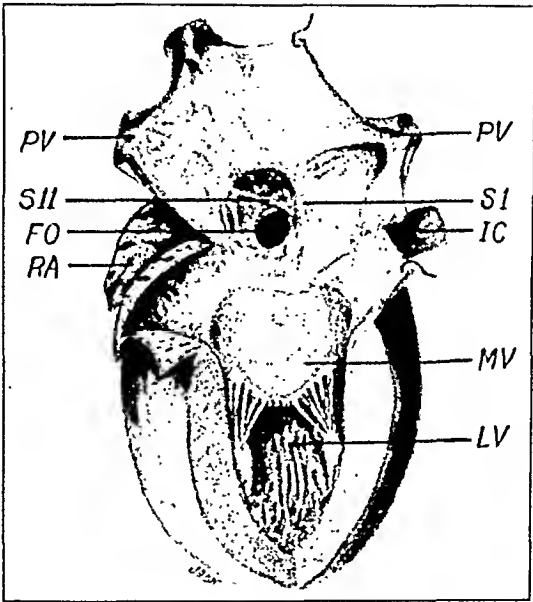
7 = Bulbar septum

8 = Interventricular septum

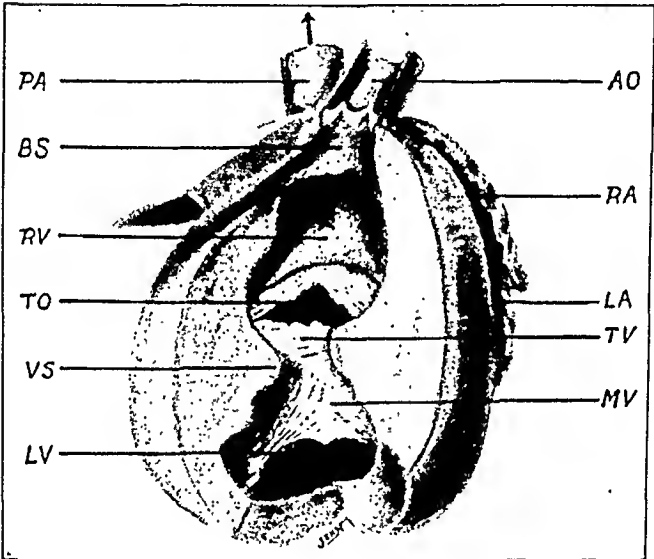
9 = Papillary muscle to mitral valve

10 = Interauricular septum

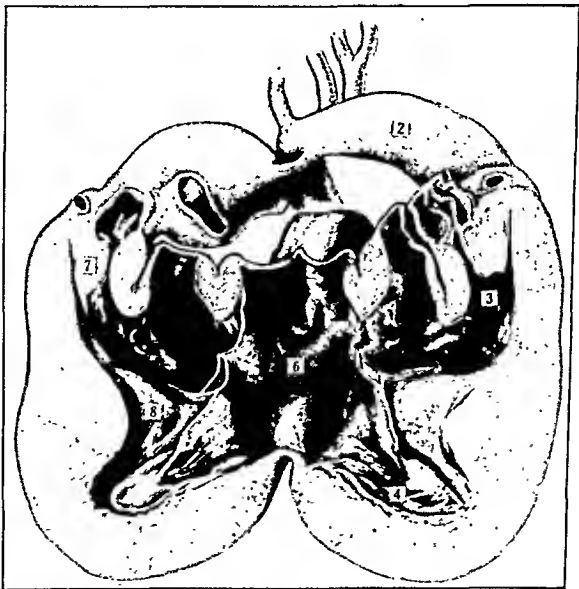
11 = Pulmonary vein



4



5



6

PLATE 49

FIG. 7. Diagrammatic representation of the distribution of the ventricular branches of the coronary artery.

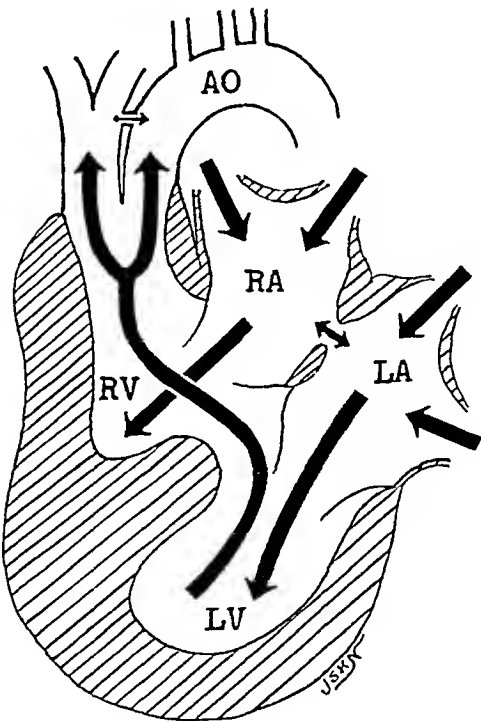
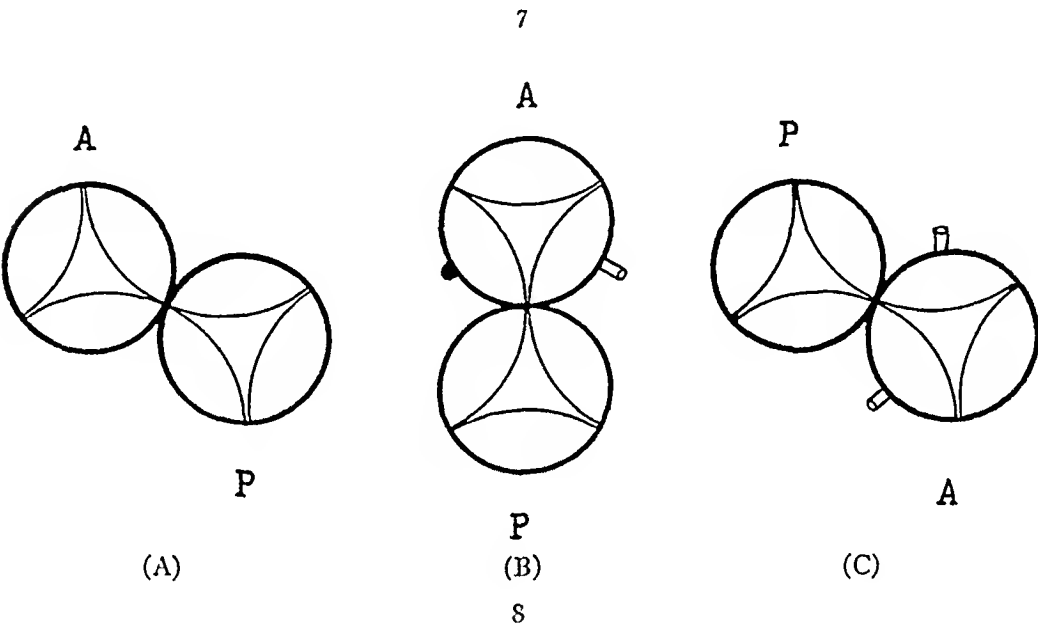
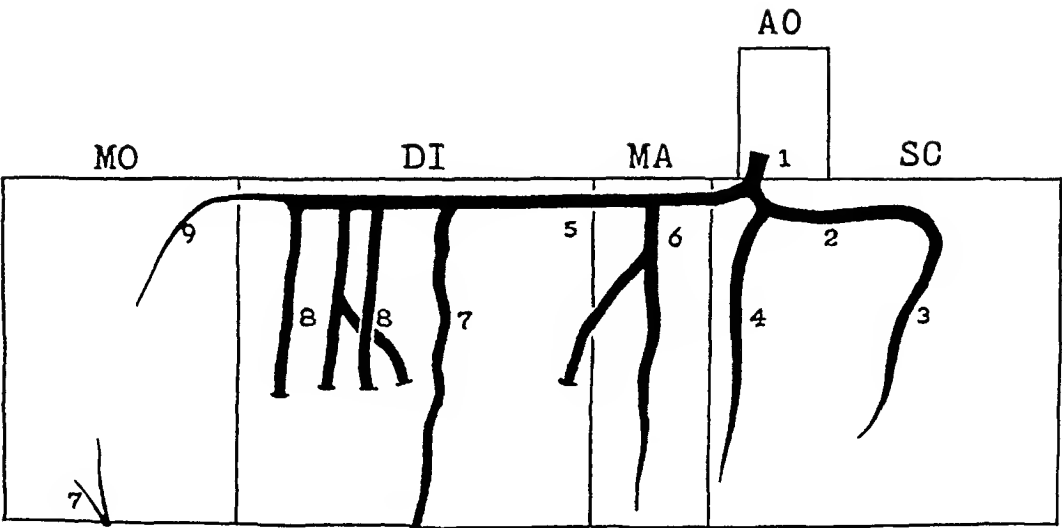
- | | |
|-------------------------------|--------------------------------------|
| 1 = Right coronary artery | 6 = Marginal branch |
| 2 = Infundibular branch | 7 = Posterior descending ramus |
| 3 = Anterior descending ramus | 8 = Posterior rami of left ventricle |
| 4 = Anterior ramus | 9 = Terminal branch of circumflex |
| 5 = Circumflex branch | |

FIG. 8. (A) = Position of aortic *A*, and pulmonary *P*, orifices in heart of early embryo.

(B) = Position of *A* and *P* in heart of author's case.

(C) = Position of *A* and *P* in heart of normal person.

FIG. 9. Diagrammatic representation of intracardiac circulation of blood.



SACROCOCCYGEAL TERATOMA *

REPORT OF A CASE

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Sacrococcygeal teratomas are reported in the literature at regular intervals. The histological characteristics determining the nature of this tumor are definite, but the exact embryological origin is still a matter not entirely clear. We present here the study of one such case that came to autopsy in the Buffalo City Hospital.

REPORT OF CASE

Clinical History: On Oct. 26, 1932, a Polish female entered the Buffalo City Hospital in the first stage of labor. In her 29 months of married life she had given birth to one normal child. There were no miscarriages. The last menstrual period began on March 29, 1932. In the latter part of pregnancy "multiple fetal parts" were noted and a diagnosis of twin pregnancy was made. The patient was one of twins and has two other twin sisters. This is of special interest when one considers the bigeminal theory of origin of teratomas. Labor lasted 18 hours and the fetus with a large sacral tumor was born dead.

AUTOPSY PROTOCOL

The autopsy was performed 27 hours after birth of the infant. The body was that of a white female infant weighing 6 pounds and measuring 34 cm. from vertex to coccyx, and 48 cm. total length. There was pitting edema of both lower extremities.

A large, globular, somewhat pear-shaped tumor mass was present over the buttocks, extending into the perineum posterior to the anal orifice (Figs. 1 and 2). This mass measured 9 by 12 cm. in its greatest diameter. On mesial section it was noted that a narrow portion near the rectal triangle was 2 cm. in diameter. The consistence of the tumor was firm and it was covered by skin which, at the upper extremity, was very thin. On section the tumor was soft, gray, friable, gelatinous and brain-like, with areas of hemorrhage. Many cystic clefts were scattered over the cut surface, some of which contained a clear straw-colored fluid. Strands of white fibrous

* Received for publication August 25, 1934.

tissue coursed irregularly over the cut surface and small, irregular, calcified fragments were encountered on sectioning. The tumor mass was found to fuse, by means of an isthmus, with a second cystic tumor mass occupying the true pelvis. This latter tumor measured 3.5 cm. in diameter and was firmly amalgamated with the rectum anteriorly, while posteriorly its capsule was loosely blended with the presacral fascia. The connection between the two masses was more like a compressed portion of one tumor, the pelvic portion compressing the rectum, vagina and urethra. The fundus uteri was elevated above the pelvic brim and the ureters, sigmoid colon and rectum were displaced anteriorly, while posteriorly the iliac veins were compressed. Definite organ rudiments or distinctive tissue elements could not be recognized in the tumor.

The peritoneal cavity contained 20 cc. of clear, straw-colored fluid. The heart was not remarkable. The lungs were atelectatic. The splenic capsule was fibrously thickened. The renal pelves were dilated and thin-walled.

MICROSCOPIC EXAMINATION

Numerous tissue blocks were made from various parts of the tumor. These were embedded in paraffin and sections stained by the hematoxylin-eosin, Unna orcein, Van Giesen, scharlach R, Spielmeyer, Holzer, and Bielschowski methods.

Tissue representatives from all three primary germ layers were recognized in the form of definite nests of squamous-celled epithelium, fibrous connective tissue, bundles of smooth muscle, cartilage, mucus-secreting, cuboidal-columnar cells arranged in small alveoli, transitional epithelium, adipose tissue and osteoid matrix. In one section an island of cartilage yielded gradually by metaplasia to fibrous connective tissue. A partly calcified osteoid matrix in another section exhibited a central reticulated area containing large, thin-walled capillaries, with small concentrations of erythropoietic cells, *i.e.* immature megaloblasts, erythroblasts and macronormocytes.

The many cysts were of varying shape and size. Their lining cells were of two types. In the larger cysts they were small, round or cuboidal, with scant cytoplasm and nuclei which were stained deeply with hematoxylin; these cells were disposed mostly in two

and sometimes three layers, the deeper ones being smaller and less differentiated. There were many papillary projections of the cellular linings. In cross-sections of the villous processes the investing cells were cylindrical or somewhat fusiform, with a single nucleus situated at their basal poles. Thin-walled, blood-containing capillaries were in intimate contact with their centripetal ends, and a fine fibrous connective tissue reticulum formed their supporting stalk. This picture was suggestive of ependyma and choroid plexus. The other type of cyst was smaller, did not have papillary processes and its cells were palisaded in layers, with clearer, lighter staining nuclei containing several nucleoli, and their cytoplasm was more abundant and irregular. The stroma around these cystic areas was a loosely meshed, eosinophilic, fine fibrillar net. This at times was quite a prominent element in many sections. The cells enmeshed in the network were of two types; one had a round, deeply staining nucleus, while the other possessed an ovoid, larger and relatively clear nucleus with a dark nucleolus. The Bielschowski stain revealed some fibrils with intimately incorporated fine brown granules. At times these cells exhibited one or two, short, conical cytoplasmic processes and in a general way suggested glia cells.

Somewhat similar cells with little cytoplasm and a large, ovoid clear nucleus with a large, eccentrically placed nucleolus were seen in another section of the tumor. In the Bielschowski silver impregnated sections some of these showed a black, single, axon-like process, and at the other pole three or four finer dendritic processes suggesting sympathetic ganglia.

The sections through the tumormass at the point of its attachment to the rectum revealed a defect involving all the muscular layers. A fibrous connective tissue filled this defect, extending apparently from the muscularis mucosae, merging gradually into the pelvic tumor described above.

The presence of derivatives from all three germinal layers classifies this tumor as a teratoma.

DISCUSSION

Sacrococcygeal teratomas are fairly well known. Pandalai, Forsyth and Stewart¹ in 1924 reported an intrapelvic teratoma in a 12 months old infant. The tumor had extended beneath the right

gluteus maximus. The dominant tissue elements were those resembling glia and choroid plexus.

Stewart, Alter and Craig² reported a sacrococcygeal teratoma in an infant aged 2 years and 11 months. This had become carcinomatous and had metastasized to the liver, lungs and inguinal lymph nodes. Sawday³ reported a postrectal teratoma in a 4 year old child. This was removed surgically and the child died a uremic death within 1 month.

Keen and Coplin⁴ in 1906 reported a sacrococcygeal teratoma in a 2 year old child. This tumor had an opening entirely through the sacrum, and communicating with this and the rectum there was a sinus which somewhat resembled a bronchus in structure.

Rosenbaum⁵ reported the successful removal of a teratoma weighing 2 pounds from a 18 day old infant. This tumor apparently originated in the sacrococcygeal region. Leopold and Phillips⁶ briefly described a retrorectal teratoma in 1906.

Davis⁷ reported the case of a newborn infant with a pelvic teratoma that had invaded the perineum.

Parin⁸ collected 16 reported cases which were considered to be congenital presacral tumors. These were reported between the years 1900 and 1909. Fifteen of them would definitely appear to be teratomas in relation to the rectum, sacrum or coccyx. He also cited 6 reported cases which were interpreted to have undergone malignant transformation with metastases.

Thompson⁹ reported the successful removal of an intra- and extracoccygeal tumor the size of a cocoanut from a 6 months old infant, with no recurrence at the end of 2 years.

Hansmann¹⁰ has reported a teratoma anterior to the sacrum in a newborn infant which, with 15 collected reported cases, he thought originated from the neurenteric canal. Hansmann and Berne,¹¹ in describing an additional case in 1932, tabulated the salient features of 25 retrorectal sacrococcygeal teratomas. These had been reported by various authors from 1924 to 1930. One, after surgical removal from a 31 year old female, recurred with metastases.

INCIDENCE

Infants born with pelvic teratomas are considered monstrosities. According to Marchand¹² (quoted by Keibel and Mall), 615 mon-

sters were found in 81,817 births. The percentage of sacrococcygeal teratomas was not stated.

Calbet¹³ found sacral tumors (all varieties) to occur once in 34,582 births. Galletly¹⁴ stated that Kiderlen in 1889 collected 122 cases of completely or partially presacral congenital teratomas from the literature.

In the Buffalo City Hospital from 1921 to 1933 there were 5424 births, 8 of which were monstrosities. Only 1 teratoma is recorded.

Ewing¹⁵ writes that one-third of fetuses with sacrococcygeal teratoid tumors are born dead, and 90 per cent of the others die soon after birth.

THEORIES OF ORIGIN

The various theories regarding the origin of these tumors include:

(A) Adami's¹⁶ theory of continued growth of totipotential cells at the inferior growing point.

(B) The unrelated individual development of a blastomere which has been isolated during segmentation of the ovum.

In regard to theory (B) Hansmann and Berne stated that disorderly growth of tissue in the presacral region occurs later in fetal life and, therefore, is a more likely cause.

(C) The extrusion of an impregnated polar body.

(D) Herrmann and Tourneux¹⁷ thought that nerve tissue was derived from a persistence of the medullary coccygeal vestige and that ependyma and neuroglia proliferation could give rise to cystic and adenomatous growths.

(E) Bonney's¹⁸ theory of inferior dichotomy of the fetal axis.

Bucy and Haymond,¹⁹ in reporting a lumbosacral teratoma associated with spina bifida, thought that it was a "twin," whose development had been partially inhibited, assuming that a "division of the ovum might have occurred at any time during the presence of the primitive streak, *i.e.* up to the fourth week following fertilization."

(F) Borst²⁰ thought that coccygeal remnants might be transplanted to the ventral side of the sacrum and give rise to teratomas.

(G) The theory of *fetus inclusio fetalis* has been referred to as the cause of sacrococcygeal teratoma by Hirst and Piersol,²¹ McFarland,²² Payne,²³ Cutler,²⁴ and Williams.²⁵ In regard to bigerminal

(*fetus inclusio fetalis*) and monogerminal origin, Ziegler²⁶ makes the following anatomical distinction: "If a teratoma can be found to contain very diverse tissue formation which, at least in part, may be identified as representing rudiments of organs, or perhaps even fully developed organs which, however, are superfluous for the individual in whom they are found, we may consider such a tumor as a heterochthonous teratoma, or as a bigerminal implantation, *i.e.*, as a rudimentary twin which is more or less completely enveloped by the well-developed twin. On the other hand, if the teratoma contains only diverse tissues and cysts whose production does not necessarily imply the existence of a second division, the tumor may be looked upon as autochthonous teratoma, or as a monogerminal implantation." Montgomery,²⁷ in a paper concerning a sacral teratoma which contained a scapula, has reiterated this principle.

In this regard it has been mentioned above that the mother of the infant in our case was a twin sister and had other twin sisters. The family history of twin pregnancies and the form of the external tumor of the infant might suggest that it was of heterochthonous origin, but both gross and microscopic study refute any such inference.

(H) Budde²⁸ has advanced the theory that the internal teratomas are genuine tumors which result from infoldings of the three primary germinal layers with the division of the blastopore. This theory could explain the origin of the various teratomas such as mediastinal, lumbosacral, sacral, cervical, and so on, which originate in the mid-line. They are most apt to arise when organs or organ systems are budding. The same author also considers that only the external malformations are rudiments of twins.

(I) Theories of origin from the fast growing structures of the caudal extremity of the early embryo. The intimate relation of the neurenteric canal, *i.e.* the channel connecting the alimentary canal and neural tube, the postanal gut and the coccygeal remnants of the neural tube are well described by Grosser, Lewis and McMurrich.²⁹ These structures are considered by many adherents to the monogerminal theory to be a fruitful source of sacrococcygeal teratomas. Middeldorpf³⁰ first attributed the origin of sacrococcygeal teratoma to the postanal gut. He described a cystic fatty tumor containing a structure resembling intestine. This was adherent to the rectum and had an external opening. Bland-Sutton³¹ also thought that

retrorectal sacrococcygeal teratomas arose from the postanal gut. Hundling³² concluded that "remains of the lower neural canal and the postanal gut appear to form the basis for many of the ventral tumors," and that all the body tissues may be represented.

Galletly included the neurenteric canal as a possible source of origin of congenital presacral tumors. Hansmann believed that the neurenteric canal or its remnants are a source of these tumors and found that they were in relation to the spinal canal, either intra- or extradural, and associated with anterior sacral defects. He recognized that tissue from the filum terminale could produce similar tumors. Hansmann and Berne implied that definite proof of origin in the neurenteric canal could be predicated upon the presence of an anterior sacral defect. Ewing supports the neurenteric canal theory in quoting the cases of Hildebrand, Jasterboff and Ritschl, and von Bergmann.

The tumor in our case did not contain organs or any well formed rudiments of organs and, therefore, it is assumed to be of monogerminal or autochthonous origin. It was large and well encapsulated, filling the pelvis, and extended out into the perineum. Anterior sacral defects were not present, nor was there any connection with the spinal cord, its terminations or coverings. Therefore, it is not likely to have originated from the neurenteric canal.

The tumor was attached deeply into the posterior wall of the rectum at a site which approximated the junction of the embryonic postanal gut with the primitive gut. The hypothesis that the tumor originated solely from the postanal gut is opposed by absence of intestinal mucosa in the tumor or its pedicle. Budde's concept of the infoldings of the primary germinal layers could be considered as explaining the complex histological picture. However, the attachment of the tumor to the rectum cannot be dismissed summarily. It is felt that in some way the postanal gut was involved in a snaring-off process with complexes of cells from other tissues. This probably occurred during the full embryological development of the postanal gut.

With the recession and disappearance of the postanal gut, which is a normal embryological process, the tumor attachment was carried into the rectal wall. In the meantime the complicated cell complexes continued to proliferate and ultimately formed the intricate teratoma found at autopsy.

SUMMARY AND CONCLUSIONS

1. A case of a large, intra- and extrapelvic teratoma in a newborn infant is presented, the gross and histological findings being reviewed in detail.
2. Some of the literature concerning the incidence and theories of genesis is briefly reviewed.
3. There are criteria suggesting that the tumor in the presented case originated in relation to the embryonic postanal gut.

NOTE: The author wishes to express appreciation to Dr. Wm. F. Jacobs for his helpful criticism of the material in this paper.

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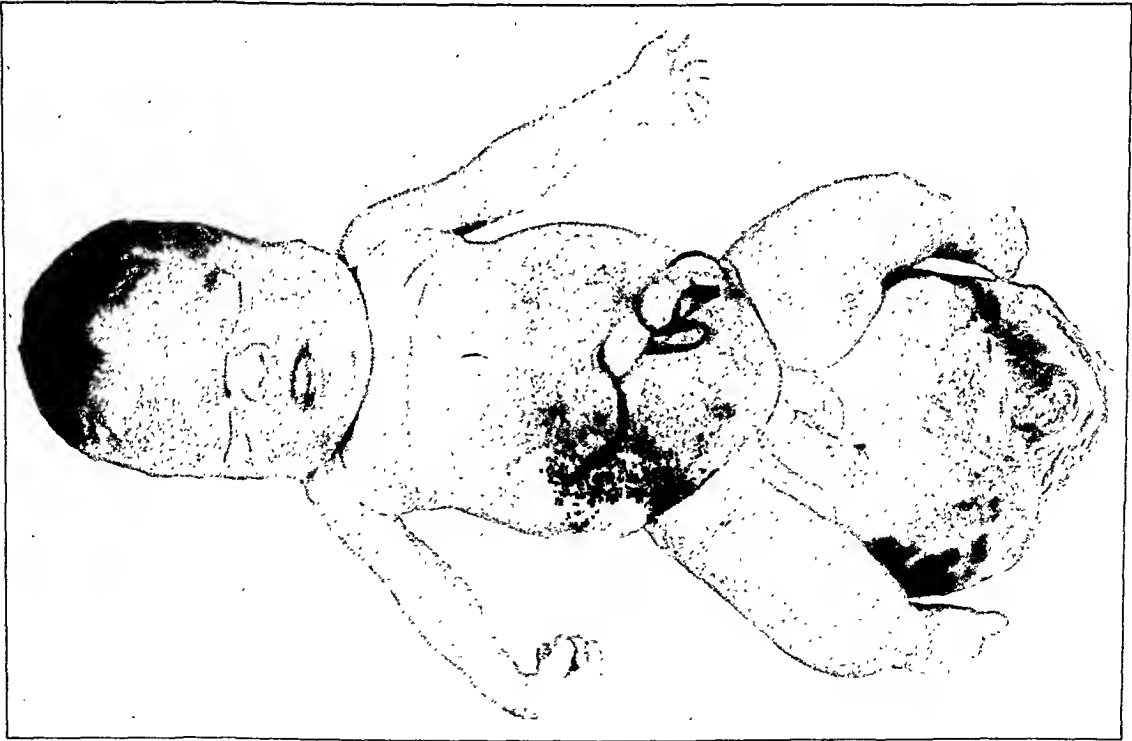
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DESCRIPTION OF PLATE

PLATE 50

FIG. 1. Anterior view of fetus showing attachment of tumor.

FIG. 2. Lateral aspect of tumor mass. Note edema of lower extremities.



INTERVENTRICULAR SEPTAL DEFECT, DEXTROPOSITION OF AORTA, AND DILATATION OF PULMONARY ARTERY *

REPORT OF A CASE WITH STRUCTURAL PATHOGENESIS

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Defects of the interventricular septum are not rare, their reported incidence varying from 225 cases in a statistical study of 850 cardiac anomalies,¹ to 5 in 753 cases.²

The combination of interventricular septal defect, dextroposition of the aorta and dilatation with hypertrophy of the pulmonary artery is "extremely rare," according to Baumgartner and Abbott.³ Abbott has christened this association of defects the "Eisenmenger complex" because it was first described by Eisenmenger in 1897.⁴ Search of the literature has revealed only one other similar case, which also has been published by Abbott.⁵ It therefore is our purpose to report this as another case of what apparently is a very unusual condition.

REPORT OF CASE

Clinical History: E. M., a white male, 10 years of age, admitted to the Buffalo City Hospital April 25, 1933, had been under observation in the hospital in both in and out-patient departments, for 4 years. His mother stated that there was transient "blueness" at birth. Since this time cyanosis and dyspnea have been manifested consistently with exercise or fright. He always has had a "shiny red complexion." Past illnesses were pertussis, measles, mumps and scarlet fever; the only operation was a tonsillectomy. There was jaundice for a week some years prior to admission.

Physical examinations made at intervals revealed the following: The right chest wall bulged somewhat anteriorly. The apical impulse, which was diffuse, was just medial to the right midclavicular line. Cardiac dullness extended 1 cm. beyond the left parasternal line. There was an apical systolic murmur and a snapping second sound in the mitral area. Moderate clubbing of the fingers was present. There was circumoral pallor, in contrast to cyanosis of the nail beds, ear lobes and mucous membranes of the mouth and lips. There were some carious teeth and gingivitis.

* This case was reported in abstract before the Buffalo Pathological Society, October 29, 1933.

Received for publication August 30, 1934.

Laboratory Studies: The red blood cell count was 5,120,000 per cmm., hemoglobin 80 per cent (Sahli). Urinalysis was negative three times and once showed 2 plus albumin and many hyaline casts.

Electrocardiograms exhibited an inverted T wave in lead I, partial inversion of the Q. R. S., and left ventricular preponderance in leads II and III. Impression: congenital dextrocardia plus an enlarged left ventricle resulting from some congenital defect other than that of location.

X-ray, 1 year prior to last hospital admission, seven foot plate: Gt. Vs. 5.8; M. L. 7.7; M. R. 4.1; longitudinal 11.8; transverse 11.8 and thoracic diameter 21.2 cm. Diagnosis: dextrocardia. Previous X-ray studies since 1929 were the same, except for smaller dimensions.

The patient had been in his usual state of health until 2 days before admission to the hospital when he suddenly felt dizzy, weak and cold. Because of extreme cyanosis he was placed in bed the next day by his mother and the following day brought to the hospital.

In the hospital he complained of soreness of the throat and slight pain in the left ear. The throat was slightly reddened and the left ear negative. He had severe attacks of dyspnea and cyanosis, and the character of the cardiac murmurs changed. The liver became palpable 2 finger breadths below the costal margin. Death occurred the third day following a particularly severe interval of cyanosis and dyspnea. The temperature varied from 99.4° to 103.8° F., the pulse from 100 to 144 per minute, and the respirations from 26 to 38.

AUTOPSY REPORT

The autopsy was performed 2½ hours after death. The body weighed 70 pounds and was 120 cm. in length. There was marked lividity of the ear lobes, lips and nails. The fingers were slightly clubbed. There were some anomalous lobes of both lungs. The spleen and liver and gastro-intestinal tract were passively congested.

Anatomical Diagnoses: (1) Interventricular septal defect, dextro-position of the aorta, hypertrophy and dilatation of the pulmonary artery, patent foramen ovale, *dextroversio cordis*, persistence of an enlarged but obliterated ductus arteriosus, anomalous great vessels. (2) Hypertrophy and dilatation of both ventricles. (3) Coronary thrombosis. (4) Passive congestion of the liver, spleen and gastro-intestinal tract.

DESCRIPTION OF HEART

The heart *in situ* was roughly quadrilateral in shape. In position it was so situated that its apex and slightly more than one-half of its presenting surface was to the right of the midline. Its long axis crossed the midline obliquely in a plane extending from the left mid-clavicular point to the middle of Poupart's ligament on the right. The base of the heart was approximately at right angles to the mid-

line. When viewed from the front the base had a pointed appearance because of the great hypertrophy of the infundibulum. The anterior surfaces of the right and left ventricles presented to an equal degree. Posteriorly the left ventricle, and some of the right, were in relation to the dome of the diaphragm.

The heart weighed 310 gm. The myocardium was firm and deep red in color. The walls of the right and left ventricles measured respectively 1.5 and 1.8 cm. in thickness. The musculature of the infundibulum was especially hypertrophied. The right ventricular cavity was approximately one-third larger than the left. The trabeculae carneae in both, especially the right, were greatly hypertrophied.

In what would correspond to the dorsal and superior part of the interventricular septum of the normal heart there was a defect measuring 3 by 2.5 cm. The borders of this were rounded. The anterior and interior parts of the septum were well formed, fleshy, and measured 1.2 cm. in thickness and 1.4 cm. anteroposteriorly. The anterior part of the septum was arched in the form of a ridge, posteriorly, superiorly and to the right, thus bounding these aspects of the interventricular foramen. This fleshy band just described was continuous with the posterior wall of the conus of the right ventricle (Fig. 3). The superior border was 1.4 cm. in thickness. Viewed thus from the right, the superior and posterior boundary of the interventricular foramen partly separated the cavity of the right ventricle from the aortic vestibule of the left; it also partly obstructed the dextroposed aortic outlet.

From the left side the interventricular foramen faced directly into the chamber of the right ventricle and upward into the aortic vestibule.

There was no membranous portion of the interventricular septum. There was the normal fibrous attachment, that is, the remnant of the embryonic aortopulmonary septum, between the first part of the pulmonary artery and the aorta. This was continued into the fleshy superior border of the interventricular foramen, where it ended abruptly (Fig. 3). The fibrous remnant of the aortopulmonary septum was continuous with the atrioventricular fibrous rings, as it normally is.

The tricuspid orifice was 8.7 cm. in circumference. There were three, well formed, normally disposed cusps. These were attached

to an enlarged papillary muscle in the floor of the right ventricle. The medial cusp was attached also by a muscular band 1.5 mm. thick to the inferior right margin of the interventricular foramen (Fig. 3), and by an elongated chorda tendinea which traversed the interventricular foramen to be attached to a small, medially situated papillary muscle on the posterior wall of the left ventricle. There was a slight amount of fibrous thickening of the posterior cusp of the tricuspid and the endocardium of the right auricle.

The right auricle was capacious. The right auricular appendage measured 6 by 6.6 cm. in its greatest diameters, and its musculi pectinati were enlarged.

The mitral valve ring measured 7.2 cm. in circumference. The cusps, chordae tendineae and papillary muscles were situated normally. The latter were moderately hypertrophied. The left auricular appendage measured 3 by 3.2 cm. in its greatest diameter; its musculi pectinati were about one-third as thick as those of the right auricular appendage.

There was a patent foramen ovale. This measured 0.5 by 1 cm. in its greatest diameter, and passed obliquely through the interatrial septum. Its borders on the right were raised and well rounded. Its left orifice was partly obstructed by two diametrically situated muscle bands (Fig. 2).

The pulmonary artery arose in normal position from the hypertrophied infundibulum of the right ventricle. The pulmonic orifice and artery respectively were 7 and 8.3 cm. in circumference, while the aortic orifice and ascending aorta, at a corresponding level, measured 4.3 and 4.2 cm. in circumference. The walls of the aorta and pulmonary artery were respectively 1.3 and 2.2 mm. in thickness.

The great arteries arising from the aortic arch were anomalous, falling somewhat into the fifth class of anomalous arteries described by Piersol. A non-patent ductus arteriosus measured 2 by 0.4 cm.

The coronary arteries were normally distributed. The posterior descending ramus of the left contained adherent thrombus.

DISCUSSION

In attempting to explain the genesis of the principal anomalies presented here, a good understanding of the embryology of the

human heart, particularly of the formation of the interventricular septum, is necessary. Practical knowledge of this sort is probably best acquired in studying the hearts of serially sectioned young human embryos. Such were used by the author recently in making Born wax-plate serial reconstruction models of the heart and endocardial cushions.⁶

The bulk of the interventricular septum is derived from the septum inferius. This appears as a myocardial ridge on the ventral caudal wall of the common ventricle about the fourth week of embryological development. It grows dorsally and cranially, the ventral and dorsal tips developing faster than the central portion, which consequently is concave toward the axis of extension. It possesses a single layer of endothelium which is continuous with that of the muscular trabeculae. The central, concave free edge of the primary interventricular septum is joined in part by the fused atrioventricular endocardial cushion mass, the proximal bulbar septum and the aortopulmonary septum. This central portion is recognized in the fully formed heart as the pars membranaceum, the undefended space, or Peacock's septum.

According to Tandler,⁷ the anterior or ventral bulbar swelling, or the prolongation of the concave lower edge of the proximal bulbar septum, becomes continuous with the ventral prolongation of the septum inferius. The dorsal prolongation, the bulbar swelling B, grows to the right of the tubercle of the anterior endocardial cushion and is broadened and flattened. The interventricular foramen at this stage is bounded as follows. "If one follows the margin of the foramen interventriculaire, beginning with the posterior prolongation of the septum bulbi, it is found to be a spiral ridge that runs upward along the free concave border of the proximal septum bulbi, passing anteriorly into the edge of the septum interventriculaire, then along this downward and backward and finally upward again, to terminate at the right tubercle of the posterior endocardial cushion. . . . The opening so bounded unites not only the two ventricles, but also leads upward and to the right into the pulmonary artery and to the left and upward into the aorta."

Tandler explains the actual closure of the interventricular foramen through downgrowth of the proximal bulbar septum to reach the septum inferius, the right ends of the fused atrioventricular cushions contributing to a degree that cannot be estimated.

Laubry and Pezzi⁸ in their lucid description add two factors to the simple mechanism of closure of the interventricular foramen described by Tandler, *viz.* thickening of the interventricular septum and a "budding" of the fused endocardial cushions.

Mall⁹ states that the aortopulmonary septum blends with the cushions through a dorso-lateral wing, which is divided to encircle the right atrioventricular orifice (*cf.* Tandler). The more cranial limb unites with the right lateral endocardial cushion, and the caudal one unites with the right lower wing of the anterior endocardial cushion. This almost complete circling of the right atrioventricular orifice in the embryo is attested in the adult heart by the blending of the septum aortopulmonale (the tendon of the conus — see Gray's Anatomy) with the right atrioventricular fibrous ring at its attachment to the medial cusp. He found that the interventricular foramen in his embryo No. 353, 11 mm., "is bounded above by the union of the septum aortopulmonale and the anterior cushion, in front by the septum aortopulmonale, behind by the extended portion of the posterior cushion and below by the muscle of the interventricular septum." As the aortopulmonary septum and the septum inferius approach, the vestibule of the aorta is shifted more and more to the left, so that when the pars membranacea septi has formed, the aortic vestibule is transferred to the left ventricle, leaving the pulmonary infundibulum in the right ventricle.

Keith¹⁰ felt that as the infundibulum of the bulbus cordis expanded, a band of muscle arising from the infundibular septum fell across the interventricular foramen and with the endocardial cushions occluded it. Careful study of serially sectioned young human embryos at our disposal failed to reveal any such bands.

Frazer¹¹ believes that the first requirement for separation of the ventricles is disappearance of the bulboventricular ridge and a portion of the adjacent bulbus and ventricular wall. It is his opinion that the interventricular foramen never disappears, but remains patent as the opening from the left ventricle into the aortic vestibule.

With the apposition of the atrioventricular endocardial cushions, the shifting of the septum inferius to the right, the prolongation of the right bulbar ridge and the ablation of the bulboventricular ridge, the axis of the aortic channel is directed toward the left ventricle, which is increasing in size. The fusing lower ends of the proximal bulbar swellings join with the free edge of the septum inferius, to

which there is accretion of the trabeculae. Through this process the bed of the aortic channel is composed of part of the dorsal atrioventricular endocardial cushion and the right tubercle of the ventral cushion. With further growth of the bulbar swellings, the cranial portion of the right atrioventricular orifice is closed and remains as a part of the floor of the aortic vestibule, only the caudal portion of the right atrioventricular orifice remains as a permanent opening. In this manner the septum membranaceum is formed, the aorta is directed into the left ventricle and the infundibulum of the bulb remains to conduct blood from the right ventricle into the pulmonary artery.

Frazer's theory, which seems the most logical, would leave one the impression that the right ventricle is the truest representative of the primary heart tube, while the left ventricle is simply a diverticulum of the former, the ostium of the diverticulum being directed from the right ventricle into the aortic vestibule.

Disparity in size of the pulmonary artery and aorta is frequently explained as the result of unequal division of the embryonic truncus and bulbus arteriosus. In this case, however, the equality of the remainder of the aorta with the ascending portion attests to an equal division of the truncus and bulbus. The cause, therefore, must be sought elsewhere.

One can only speculate as to which factor involved in the closure of the interventricular septum has been defective here. The presence of normally disposed atrioventricular fibrous rings, valves and the intact lower part of the interatrial septum should be predicated on the fulfilment of most of the growth functions of the endocardial cushion material. The fibrous union between the roots of the ascending aorta and pulmonary artery, which is here continued into the anterosuperior crescentic margin of the muscular interventricular septum, would also suggest that the aortopulmonary septum had developed but failed to reach the interventricular foramen. However, such failure alone would not likely give rise to such a large opening. If one follows Frazer's theory, the large interventricular foramen could be explained as failure of the lower part of the bulbar ridges to fuse. If such occurred, dextroposition of the aorta would result from failure of the aortic vestibule to be shifted to the left.

The partial obstruction of the aortic vestibule by the posterosuperior border of the interventricular foramen would tend to cause

blood to be shunted from the left ventricle into the right, from whence its reflux would be partly prevented by the relation of the medial tricuspid cusp to the interventricular foramen. The disposal of an increased quantity of blood by the right ventricle would lead to its dilatation and hypertrophy, and also dilatation and hypertrophy of the pulmonary artery. In this respect the condition is much like that described by Abbott,³ who has written " . . . for the pulmonary conus in the case here described is roomy and thick-walled, and leads into a very large and greatly dilated pulmonary artery (8.5 cm. wide). Both conus and artery have here developed as in the normal heart, and have evidently undergone dilatation to accommodate the additional volume of blood received from the left ventricle through the defect in the left to right shunt that prevailed owing to the relatively higher pressure in the left ventricle, while the dextroposed aorta received a mixed current of venous and arterial blood from both ventricles above which it rides and transmits to the systemic circulation, producing a raised oxygen — unsaturation in the capillaries there with resultant cyanosis." The dextroposed aorta was in a position to receive some blood from the right ventricle. The amount of venous blood in the systemic circulation would tend to be increased with increase of pressure within the right ventricle. This would account for cyanosis during exercise.

SUMMARY AND CONCLUSIONS

1. The rare combination of interventricular septal defect and hypertrophy and dilatation of the pulmonary artery is presented and described.

2. The theories concerning the formation of the interventricular septum are reviewed. Of these, that advanced by Frazer¹¹ affords a reasonable explanation of the defects in this case.

NOTE: The author wishes to express appreciation to Dr. Wm. F. Jacobs, Pathologist, Buffalo City Hospital, for helpful criticism, and to Dr. W. J. Atwell, Professor of Anatomy, University of Buffalo Medical School, for his kind assistance and loan of serially sectioned human embryos from his collection.

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DESCRIPTION OF PLATES

PLATE 51

- FIG. 1. Anterior aspect of heart and lungs. Note the partial turning of the heart to the right, its rhomboidal shape and the prominent pulmonary infundibulum. The anomalous lung lobes are also apparent.
- FIG. 2. The interventricular septal defect, as seen through the opened left ventricle. Note that the chordae tendineae of the medial cusp of the tricuspid valve are attached in the floor of the defect. The hypertrophy of the left ventricle wall is evident.



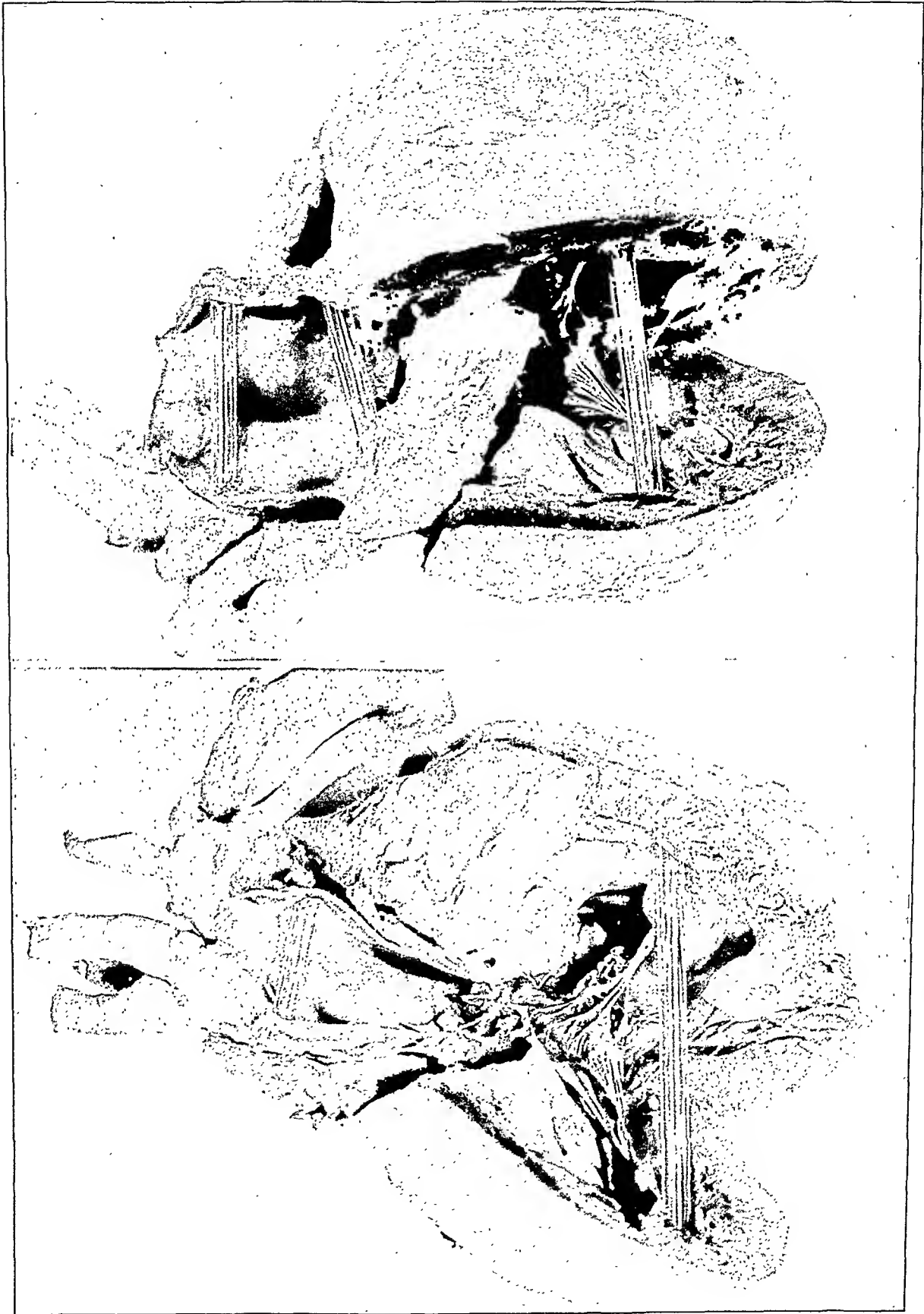
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PLATE 52

FIG. 3. Interventricular septal defect viewed through the opened right ventricle.

The hypertrophy of the musculature of the right ventricle and pulmonary infundibulum is seen. Note the dextroposed aorta and the obstructing, thick posterior wall of the pulmonary infundibulum. There is coarctation of the arch of the aorta. Compare the size of the ascending aorta with that of the corresponding portion of the pulmonary artery in Fig. 4.

FIG. 4. The right ventricle is opened and the walls of the pulmonary infundibulum spread apart. Compare the diameter and thickness of the pulmonary artery with that of the aorta in Fig. 3.



A MALIGNANT HEMANGIOMA OF THE LUNG WITH MULTIPLE METASTASES *

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Malignant hemangiomas with metastases by way of the blood stream are extremely rare. Wright,¹ in 1928, described such a case and stated that less than a dozen reports of proved cases of this group could be found in the literature. The same statement is true today. He reviewed briefly the reports of the following authors: Borrmann,² Ewing,³ Shennan,⁴ Homans,⁵ Langhans,⁶ Theile,⁷ and Jores.⁸ Attention was called to the fact, however, that of these seven reports only the last three qualified as regards malignancy of the primary tumor. Wright's case should be classed with those of Langhans, Theile and Jores. Dassel's ⁹ case should also be included in the latter group.

Schlopsnies,¹⁰ in 1930, gave a fairly complete summary of the reported cases of multiple angiomatous tumors. The summary includes multiple benign angiomas, angiosarcomas, multiple "system" tumors and *malignant metastasizing hemangiomas*. He classified his own case as an angiosarcoma of the spleen with multiple foci confined apparently to the hemopoietic system. In addition to this case, the reports of Grabowski,¹¹ Shennan,⁴ and Wollstein¹² should be included among the hemangiomas with multiple foci but histologically benign. It is somewhat doubtful whether or not the angiosarcomas should be included in the group of malignant hemangiomas, since many of these may be only vascular sarcomas and not true tumors of the vascular system.

REPORT OF CASE

Clinical History: L. P., a married, white female, aged 40 years, was seen by a physician on Nov. 28, 1933, at which time she complained of severe pain, of 6 weeks duration, in the right hip; backache, the onset of which was concurrent with the last menstrual period 6 weeks previously; weakness, insomnia, mental

* Received for publication August 29, 1934.

depression and nausea of 1 weeks duration, with some vomiting. She had had influenza in 1918, "heart trouble" and rheumatic fever at 16 years of age.

Her father had hypertension, and her mother had a manic depressive psychosis. Her brothers and her husband are living and well. During the past 18 months the husband had lost his business and at the time of the patient's illness necessities were being provided by other members of the family. Three pregnancies had resulted in three normal deliveries. The children, aged 16, 9 and 6 years, are normal.

Physical Examination: This revealed a pale, but well developed, well nourished woman of about 40 years. The positive findings were mitral stenosis and regurgitation, pulse 136, blood pressure 105/88. There was a tiny cervical polyp protruding slightly from the cervical canal.

Examination of the blood showed a hemoglobin of 40 per cent, red blood cells 3,100,000, white blood cells 5600, with the following differential count: polymorphonuclears 60, lymphocytes 31, monocytes 4, eosinophils 2, and basophils 2.

The patient was exhausted by the examination and wept most of the time. Her physician advised rest in bed, preferably in a rest home or sanitarium with supportive treatment. Bromides were prescribed as a sedative.

Course of Illness: On Dec. 2, 1933, the patient was cheerful, was eating well and nausea had disappeared. The pain in her hip was relieved. The blood pressure was 120/84, the pulse 90. Improvement continued until December 17th. At that time the hemoglobin had risen to 48 per cent and the red blood cells were 3,900,000. The pulse had dropped to 88.

From Dec. 17, 1933 to Jan. 7, 1934, there was recurrent pain in the right hip, for which it was necessary to use codein, as salicylates failed to relieve the pain. On January 7th, at 2 A.M., the patient complained of a severe sharp pain in the right chest. She was sent to a private hospital where she remained for a week. Examination revealed a small area of consolidation at the base of the right lung. There were râles over this area and increased density of the mediastinum. The patient was dyspneic. The temperature was 100.4° F., the white blood count 5800. It was believed by the physician in charge that the patient had developed pneumonia, possibly tuberculous in origin. While in the hospital the patient's temperature fluctuated from 101.8° F. to normal.

On January 13th the patient returned home. Her condition continued steadily downward. Three days before death, the physician was permitted to call a cardiologist and a surgeon in consultation. A diagnosis of acute cardiac decompensation, internal hemorrhage and shock was made. The patient's physician had previously made a tentative diagnosis of a malignant pelvic tumor with pulmonary metastases.

The patient was admitted to the Los Angeles County Hospital where examination revealed a pale, slightly yellowish, dyspneic and extremely weak, white female. There was flatness over the chest from the right base to the second rib. Breath sounds were distant or absent. The left lung field was resonant. The heart was displaced to the right, the rate rapid but regular. Blood pressure at this time was 80/40. Examination of the abdomen showed that the liver was displaced downward about 1 inch below the costal border. No masses and no tenderness were found.

It was thought that hemorrhage had taken place into the right pleural cavity, probably from pulmonary metastases. A thoracentesis yielded 200 cc. of bloody fluid, and 150 cc. of air was replaced.

A blood examination showed 30 per cent hemoglobin, 2,380,000 red blood cells and 14,700 white blood cells. The urine was essentially normal.

While preparations were being made for a transfusion the patient died suddenly.

AUTOPSY REPORT

The autopsy was performed $3\frac{1}{2}$ hours postmortem. The body is that of a well developed, well nourished white female about 40 years of age. The skin and mucous membranes are pale. The superficial lymph nodes are not palpable and there is no edema. The diaphragm level is at the fifth rib on the right, the sixth rib on the left.

On removing the sternum about 3000 cc. of bloody fluid is found in the right pleural cavity and about a liter of somewhat bloody fluid is found in the left pleural cavity.

The pericardium is normal. The heart weighs 240 gm. and is normal, except for the right coronary which has no opening into the aorta, although the artery, which is smaller than normal, is found in the epicardial fat about 1 cm. from the aorta. There is a small dimple in the aorta opposite the right aortic cusp at the usual site of the coronary orifice.

The right lung is almost completely collapsed; the left lung is partially collapsed. The right lung weighs 330 gm. and forms a mass a little larger than a man's fist (Fig. 1). All three lobes are quite firm, except a small portion of the upper lobe which contains air. In the pleura of the interlobar and posterior surfaces of the upper lobe, and throughout the middle and lower lobes, are a number of raised, reddish black tumor nodules which appear to contain blood. These vary from 0.2 to about 1 cm. in diameter. In places, several of these nodules are more or less confluent. At the lower border of the lower lobe there are several larger, granular red areas covered more or less with a buffy coat of fibrin. The largest of these measures 5 by 1.5 cm. These are angiomatous tumor masses with some hemorrhage into the pulmonary tissue surrounding them. There are approximately 50 hemorrhagic nodules scattered throughout the pleura. On the cut surface an occasional similar nodule is seen deep in the lung tissue, but the greater number are pleural and subpleural.

The left lung is slightly larger than the right, but weighs only 290 gm. About one-third of the upper lobe and a small portion of

the upper part of the lower lobe are air-containing. Hemorrhagic nodules similar to those described are found throughout the surface of the lung but they are not so numerous as in the right lung. In the diaphragmatic surface of the lower lobe there is a larger tumor measuring 2 by 1.5 by 1.5 cm. Occasional hemorrhagic areas are found on the cut surface. Some 20 to 25 pea-sized, hemorrhagic tumors are found scattered over the parietal pleura of the right side and 12 to 15 similar ones on the left. These are localized over the intercostal muscles.

The spleen weighs 120 gm. The capsule is smooth, the pulp pale red with distinct markings. Two small accessory spleens are found near the hilum which measure 0.6 and 1.2 cm. respectively.

The adrenal glands and kidneys are normal.

The uterus is slightly enlarged. The tip of the cervix is rather large with a smooth surface. There is a tiny polyp projecting from the cervical canal. The uterine cavity is normal. The ovaries are slightly cystic. The tubes are normal.

The duodenum, bile ducts, stomach and pancreas are normal except for marked pallor.

The liver weighs 1800 gm. In the anterior portion of the liver a number of grayish red, poorly defined, metastatic nodules may be seen through the capsule. On the under surface, just to the right of the gall-bladder, there is an elevated nodule 2 cm. in diameter, which is dull red with a few pale grayish areas showing through the red background. On the cut surface the nodules vary from 0.3 to 1 cm. in diameter, and show the same mottled reddish gray color. The remaining portion of the liver is pale and the markings are rather indistinct. The gall-bladder and contents are normal.

There are a number of small, hemorrhagic tumors in the fat surrounding the pancreas. None is found, however, within the body of the gland itself. Immediately below the head of the pancreas there is a firm, rounded mass measuring 8 cm. in diameter and consisting of a group of 12 to 15 enlarged hemorrhagic lymph nodes surrounded by fibrous fatty tissue. The largest of these nodes measures nearly 2 cm. in diameter. Most of them are reddish black, due to the large amount of blood present. Several, however, show small grayish spots scattered through them, while the more cellular ones contain considerable grayish tissue. Similar enlarged nodes are found on either side of the aorta almost to the

bifurcation. The aorta is somewhat smaller than normal. Occasional yellowish streaks are seen in the intima.

The bone marrow of the sternum is pale red and of normal consistence.

The dura and leptomeninges are normal. The convolutions over the convexity of the brain appear slightly flattened. The brain tissue is exceedingly pale. The large vessels at the base of the brain are distinctly hypoplastic and show an occasional atheromatous spot.

A rapid frozen section through one of the nodules from the lung and also one from the liver revealed cellular angiomatous growths showing malignant characteristics.

Anatomical Diagnoses: Malignant hemangioma of right lung; malignant hemangioma, metastatic, left lung, parietal pleura, liver and retroperitoneal lymph nodes; fatal hemorrhage into right pleural cavity; anemia, secondary; congenital malformation of heart (imperforate right coronary); hypoplasia of aorta and cerebral vessels, and multiple accessory spleens.

HISTOLOGICAL EXAMINATION

Retroperitoneal Lymph Node: The body of the gland is surrounded by a dense fibrous capsule. The lymphoid tissue is completely replaced by a vascular tumor consisting of a cavernous part and a more cellular part. In the cavernous part are many large spaces filled with red blood cells. These spaces are lined for the most part by the usual flattened endothelial cells. The partition walls are rather thin in most places, but occasionally spread out into fairly broad fibrous bands. An increased proportion of polymorphonuclear leukocytes is seen in the spaces among the red blood cells.

In the cellular part the blood spaces are small and partially or wholly collapsed. Only a few of the open ones contain red blood cells. The cells lining these spaces are very atypical. Some are more or less elongated, hypertrophied endothelial cells, while others are large polygonal cells with oval or spherical nuclei, many of which are hyperchromatic (Fig. 3). All gradations between these two extremes may be seen. Surrounding the spaces, or in the more cellular areas, the same kind of cell is predominant. Mitoses can be found in nearly every high power field. In many places the

stroma is loose and edematous with abundant fibrin present and considerable numbers of polymorphonuclear leukocytes.

In some of the lymph nodes the tumor tissue is arranged in whorls and papillary extensions (Fig. 2). The lymphoid tissue is almost wholly replaced by a turbulent-appearing tissue. There are numerous smaller and larger blood spaces, some of the latter forming long sinuous channels among islands and promontories of cellular tissue.

Many nucleated red cells, mostly normoblasts, are found in the vascular spaces. In one small space practically filled with normoblasts seven cells were counted. Many of these cells show the nuclear material broken up and scattered through the cell as dark staining fragments. Occasional megaloblasts are seen also. In addition to the above, many polymorphonuclear neutrophilic leukocytes are present, also moderate numbers of lymphocytes, a few eosinophils and occasional basophils.

Lung (Right): Sections through one of the vascular tumors at the base of the right lung show a number of large cavernous spaces in the pleura. Cellular, moderately malignant areas, similar to those described, border the larger spaces. The partition walls of the large sinuses are broken down in some instances and the blood is clotted. Over the surface is a layer of fibrin of irregular thickness. It was evidently from these large, coalesced blood sinuses that hemorrhage occurred into the pleural cavity.

Moving inward from the periphery one sees a peculiar arrangement. The blood spaces are relatively small, many of them collapsed. The open ones contain red blood cells and groups of extremely large phagocytic "dust cells," the latter containing either red cells in various stages of disintegration, or coarse clumps of brown pigment (hemosiderin). The septa are thick and quite vascular. Large, swollen polygonal cells line the spaces. The nuclei are large, oval or spherical, and some are hyperchromatic. These areas, no doubt, represent collapsed lung in which small hemorrhages have occurred because of the presence of metastatic tumor. In addition, there is active phagocytosis of red blood cells and all the alveolar lining cells appear to be hypertrophied.

The bronchi are nearly all large and irregular. The lining epithelial cells show hypertrophy and crowding. Many of the nuclei are pyknotic.

Liver: The nodules in the liver are roughly spherical and fairly well circumscribed growths having the characteristics of metastatic rather than primary tumors. These consist in part of angiomatous and cavernous spaces filled with blood and partly of very cellular areas similar to those described above. The angiomatous portion gradually merges into the cellular, more malignant areas. In some of the latter there is a tendency to form a papillary type of growth. In one place a medium sized vein (3 mm. greatest diameter) shows tumor growing completely through the wall at one side. Tumor is heaped up on the inside of the vessel and spreads along the wall for some distance on both sides. At one end of the same vessel a similar phenomenon has occurred. The liver cells are swollen, the cytoplasm is finely granular and pale staining. The Kupffer cells are normal.

Spleen: The capsule is of uniform thickness and the trabeculae are moderately thick in places. The reticulum is everywhere thickened and appears to be somewhat edematous. Large, early fibroblastic cells are also present. The littoral cells of the venous sinuses are large, with round or plump oval nuclei. Occasional, large, atypical tumor cells are found free in the sinuses. The lymphoid follicles are rather small. The malpighian arteries are moderately thickened and show some hyaline degeneration.

Kidney: Essentially normal.

Pancreas: Normal.

Bone Marrow (Sternum): Stained with Giemsa, many myelocytes, a moderate number of eosinophilic myelocytes and many normoblasts are seen. The red cells show moderate anisocytosis and poikilocytosis and tend to be small. Occasional megakaryocytes are seen, and now and then a large irregular cell with oval nucleus, which is probably an atypical endothelial cell from one of the malignant areas.

COMMENT

Wollstein,¹² in 1931, reported a malignant hemangioma of the lung in a female infant 4 months and 20 days old. She considers this the first case reported in which the principal tumor was found in the lung. Although the lung was the organ chiefly involved, it is not certain that this was the primary site. Shennan⁴ presented an unusual case, a female, 23 years of age, who had a large angiomatous

tumor nodule near the root of the left lung. A larger mass involved the superior mediastinum. Because of the greater size of the mediastinal tumor and the peculiar barking cough suggesting mediastinal pressure, the author favored the mediastinum rather than the lung as the primary site. The spleen was enlarged by the tumor growth, and other tumors were found in the thymus, pleurae, mediastinal lymph nodes, liver and bone marrow. In Shennan's case the tumors were largely confined to the hemopoietic system, as in most of the "multiple foci" tumors. The tumors were histologically benign, further evidence in favor of a system disease neoplasm. The patient died of hemorrhage into one of the pleural cavities.

In the author's case a similar situation is seen in regard to the primary tumor as in the 2 cases referred to above. The marked dyspnea, the fatal hemorrhage and the more extensive growth of the tumor in the right lung point to this organ as the seat of the disease. On the other hand, the metastases in the lungs, pleurae and liver are more readily explained by assuming the primary growth to have been located in the retroperitoneal lymph nodes. The tumor-like mass found in the latter situation, together with the history of pain in the right hip for a period of 6 weeks, are further points in favor of this location. It is evident that no definite conclusion can be reached in regard to the primary site.

Regardless of our inability to designate the original growth with any degree of certainty, it is quite evident that the greater number of angiomatous nodules found in the lungs, pleurae, liver and lymph nodes are true metastases. Let us consider the nodules in the liver in this respect. The tumor masses all tend to be spherical and the surrounding liver tissue is normal except for occasional small hemorrhages. The Kupffer cells particularly show no abnormal changes. In the liver metastases both cavernous and cellular angiomatous structures are found. The malignant character is evident, not only by the anaplasia of the tumor cells, but also by invasion of the wall of one of the veins.

From the foregoing, there would seem to be no escape from the conclusion that the multiple tumor foci in this case are true metastases. The characteristics that distinguish a system disease tumor are largely lacking. Although the liver and the retroperitoneal lymph nodes are involved, the spleen is not involved and, so far as we know, the bone marrow likewise is free. Furthermore, the

distinctly malignant appearance of the tumor growth wherever encountered is contrary to the usual benign histological character of the system disease tumors.

The presence of congenital anomalies of the circulatory system in this patient may have etiological significance. Cohnheim's theory of tumor growth in aberrant or misplaced portions of organs or systems, which is well established for certain kinds of tumor, suggests itself as a probable causative factor. A definite congenital anomaly was present in the right coronary artery which failed to arise from the aorta in the usual way. A small dimple was present at the usual site opposite the right leaflet of the aortic valve. Lack of any evidence of syphilitic changes and the presence of only very early atheromatous lesions in the aorta make it quite certain that the condition is congenital. The aorta was moderately hypoplastic and the large cerebral vessels at the base of the brain were distinctly smaller than normal. Two small accessory spleens were found at the hilum of the main organ.

SUMMARY

1. A case of malignant metastasizing hemangioma, of which there are less than a dozen true cases in the whole medical literature, is reported.
2. The largest tumor was found in the right lung and death was due to hemorrhage into the right pleural cavity.
3. True metastases consisting of both cavernous and malignant cellular areas were found in the lungs, pleurae, retroperitoneal lymph nodes and liver.
4. The type cell is the endothelial cell which forms blood-vascular spaces in all the tumor nodules. The cells vary from practically normal appearing cells lining the cavernous spaces to extremely large atypical cells that almost fill the blood spaces in the more cellular areas. In the latter the growth is rapid and apparently highly malignant.

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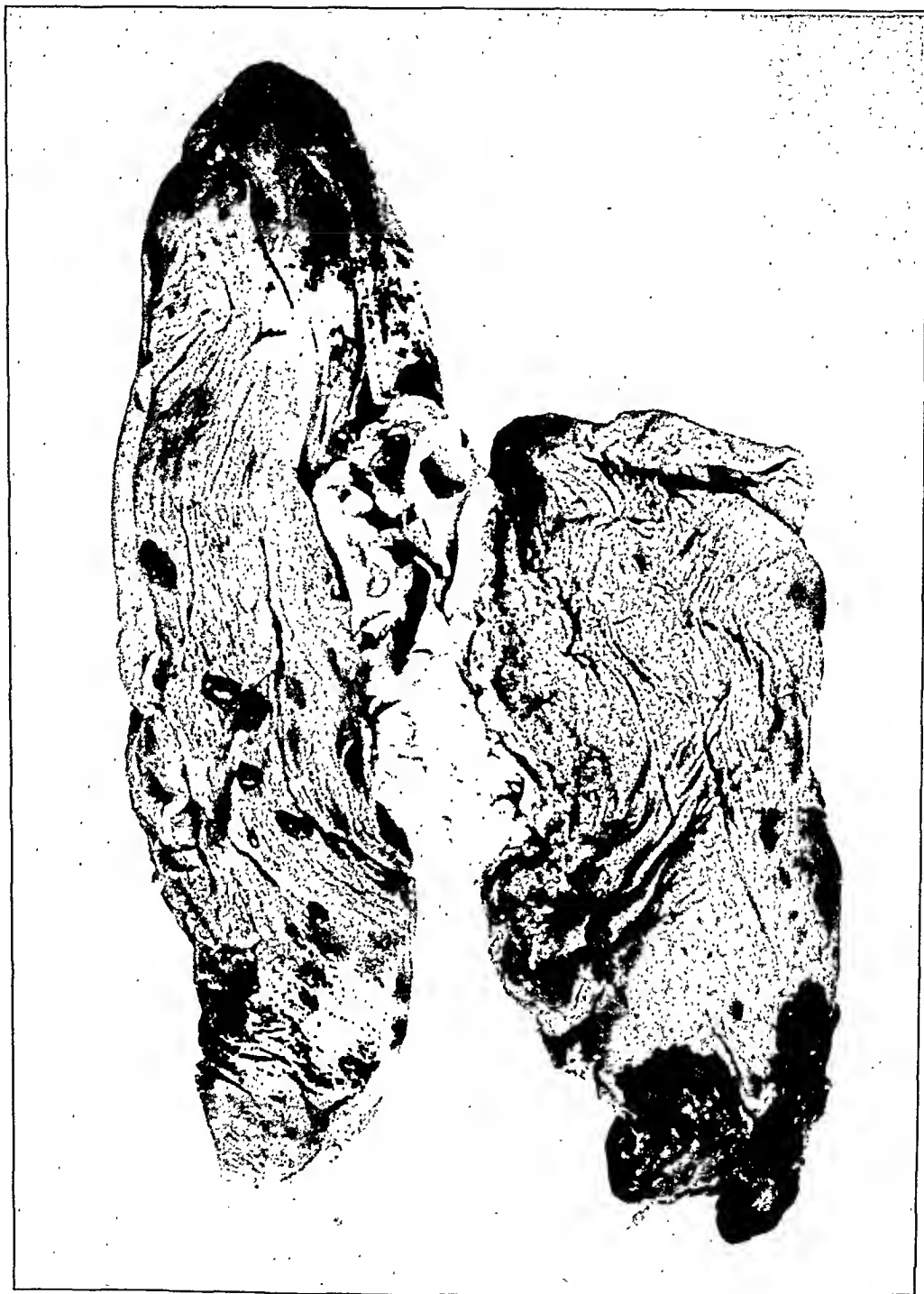
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DESCRIPTION OF PLATES

PLATE 53

FIG. 1. The collapsed right lung (two-thirds normal size) showing the main tumor mass at the base of the lower lobe with hemorrhage into the surrounding tissue. A number of hemangiomatous nodules are seen in the pleura of the upper lobe, also along the lateral border of the lower lobe.

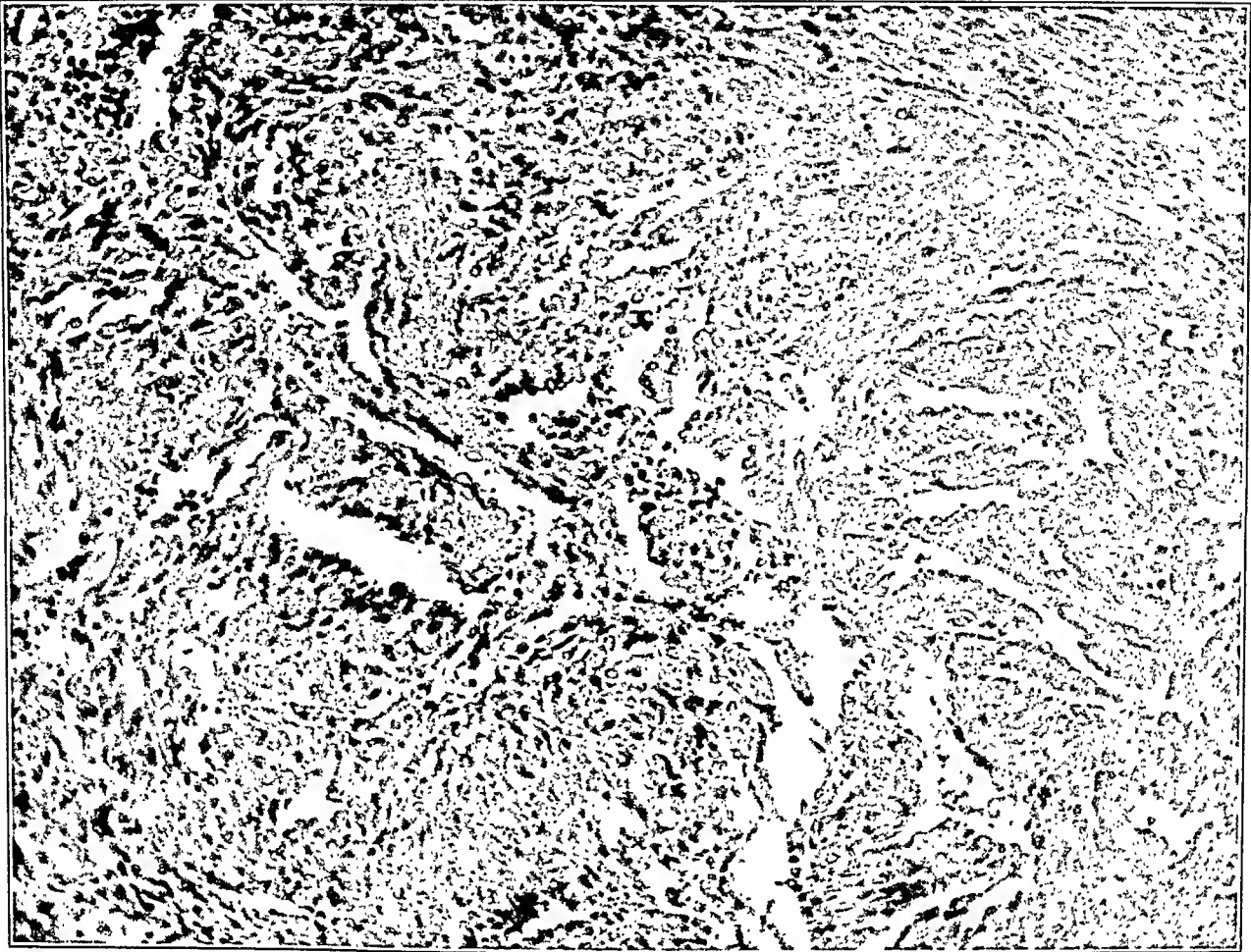


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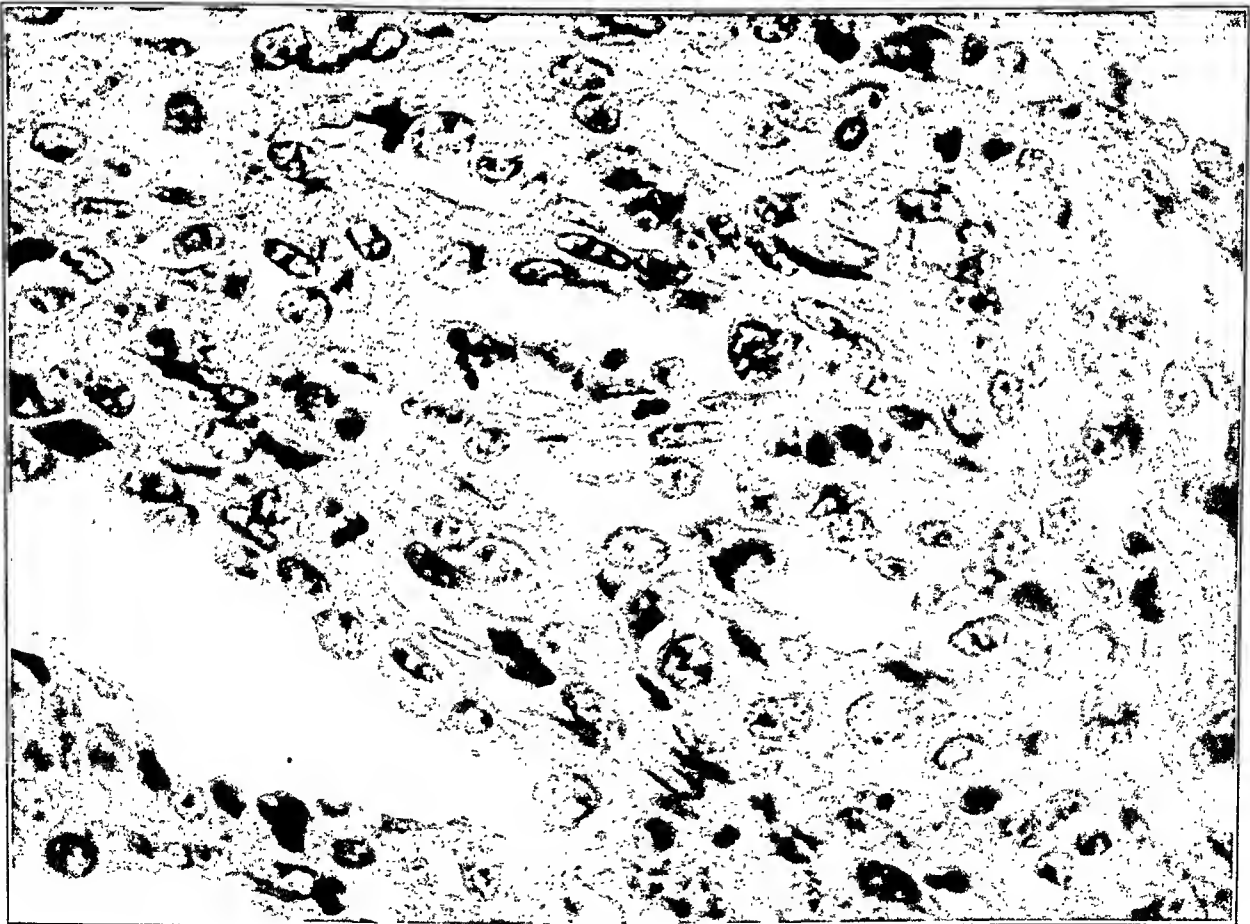
PLATE 54

FIG. 2. Photomicrograph from section of retroperitoneal lymph node showing turbulent papillary type of growth completely replacing the lymphoid tissue. $\times 160$.

FIG. 3. Photomicrograph from section of retroperitoneal lymph node showing marked anaplasia of the endothelial cells. Several mitotic figures are present in this field, but not all are in true focus. $\times 700$.



2



3

ANTIGROWTH EFFECT OF LIPOID FRACTIONS OF TISSUE EXTRACTS *

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Under the relatively simple conditions that prevail in the cultivation of cells *in vitro* it has been fully established that certain constituents of the tissues and fluids of the body stimulate growth and that others inhibit it. Carrel¹ noted that the growth of connective tissue was more abundant in the plasma of young chickens than in that of older ones and, in 1921, Carrel and Ebeling² determined that the influence of age, as reflected in the serum of the animal, was exerted not by variations of growth-accelerating factors, but by fluctuating inhibiting agents. Later, in 1923, the same authors³ separated the serum into a growth-activating and a growth-inhibiting fraction by precipitating the former by bubbling carbon dioxide through the diluted serum. Actually to accomplish indefinite multiplication of fibroblasts in artificial mediums it is required that the inhibiting effect of the plasma be counterbalanced by the addition of positive stimuli, especially in the form of embryonic juice or primary protein derivatives.^{4, 5, 6} Very potent growth stimuli are present in the tissue of Rous chicken sarcoma. Sittenfield and Johnson⁷ showed that the tumor-producing substance could be neutralized by mixing an active filtrate with blood, and subsequently recovered by buffering the mixture to a pH 4.0 and then extracting the resultant precipitate at a pH of 8.0 to obtain the active tumor agent in solution. This substance that neutralized the tumor principle was later found by Sittenfield, Johnson and Jobling⁸ to be confined to the globulin fraction of serum. Information concerning the growth regulatory mechanism which operates in the living animal has been slower to appear. So far as is now known types of growth such as embryonal, regenerative, compensatory and even neoplastic, may take place in response to stimulating and re-

* Aided by a grant from the Committee on Grants-in-Aid of the National Research Council.

Received for publication September 14, 1934.

tarding influences analogous to those of the artificial culture. Most of the investigative successes have been concerned with inhibiting agents. Certain of the hormones, when administered in excess of the normal requirements, inhibit multiplication of the cells that produce them. Loeb ⁹ found that thyroid feeding of partially thyroidectomized animals inhibited regeneration of the thyroid remnant. It has been shown by Cameron and Carmichael ¹⁰ that thyroxin diminishes not only the growth rate of the thyroid gland but of the body as a whole. In our experiments with parathyroid hormone ¹¹ and insulin ¹² the hormones in excess of normal requirements were supplied to the animal. Such excesses cause departures from the normal metabolism of the animals and the decreased karyokinetic rates observed by us may have been secondary to disturbed cell nutrition.

It was considered desirable to examine tissues not known to produce internal secretions for the presence of inhibiting agents. The kidney was chosen because of its known quick regenerative response after unilateral nephrectomy and its great growth activity in rats during the first month of life. It was readily demonstrated that the kidney contained inhibitory substances which could be extracted by dilute acids and by acid alcohol.¹³ A quantity of the acid alcohol extract equivalent to about 2 gm. of the fresh kidney was found to inhibit tubule cell proliferation. In experiments described below, evidence is presented to show that the chief inhibiting agent is of a lipoid nature, that its distribution is probably wide, since it was demonstrated in several tissues, and that it inhibits mitotic proliferation, not only of the kidney but also of the liver.

METHOD OF BIO-ASSAY

The technic of determining the proliferative activity of the renal epithelium is now essentially the same as that described in the earlier report.¹³ With the number of litters tested well above 100, it has been found that the mother rats should be kept upon an adequate commercial mash reinforced by alternate daily feedings of cheese, fresh meat or fresh cabbage and during the winter months with dry brewer's yeast. Litters 18 to 21 days of age are best and at least two litter-mates from each litter should be reserved for controls. The young rats were injected intraperitoneally and killed 24 hours

later. From the formalin-fixed right kidney there was cut a 3 mm. transverse block midway between the two poles. From the center of this block, embedded in paraffin, 8 micron ribbons were cut for staining in hematoxylin and eosin. The mitoses of the tubular epithelium were counted in two or three sections and, if the variations were considerable, several additional sections were examined.

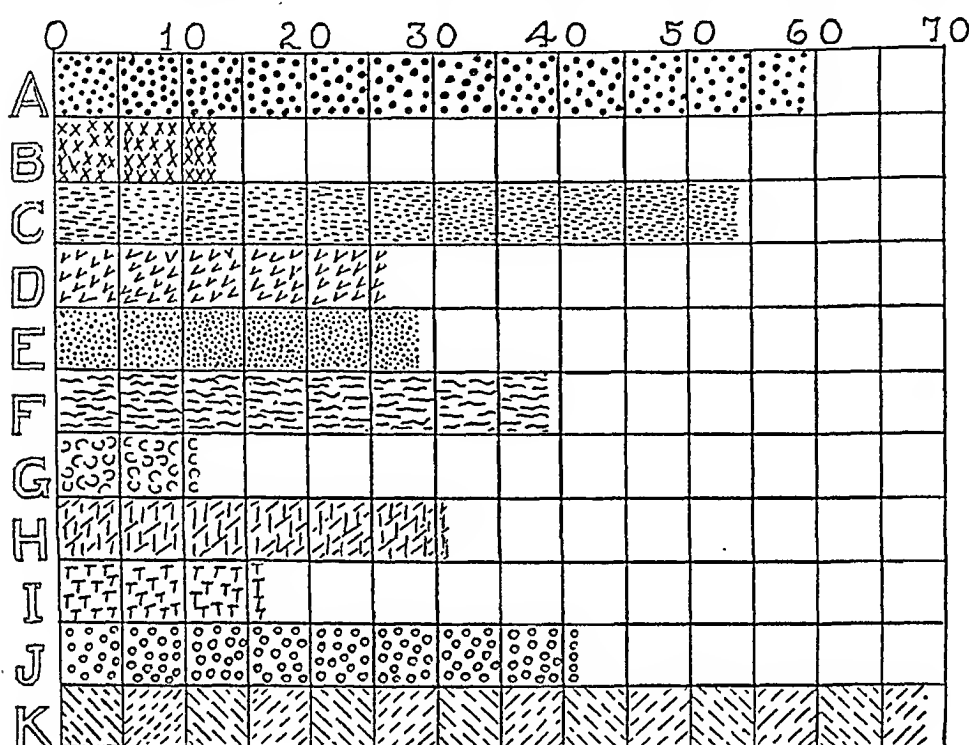


CHART I

A-K inclusive represent doses of lipoid-containing extracts which are described in the text. All doses were converted to the equivalent amount of fresh tissue represented by the extracted material. The percentages from 0 to 70 represent the reduction of the mitotic rate per gram of fresh tissue and are based on results of two or more litter-mates injected with the extract.

Although the procedure gave much uniformity, little or no weight was given to minor decreases in the mitotic rate.

LIPOID NATURE OF THE INHIBITING AGENT OF TISSUE EXTRACTS

In several preliminary experiments there were obtained indications of acute inhibitory effects following the administration of fresh tissues. Fresh macerated rat kidney was thoroughly mixed with 2 volumes of physiological saline solution and after standing 5 minutes the coarse particles were removed by straining through

muslin and centrifuging at low speed. The cloudy supernatant liquid rich in suspended cells gave marked inhibition ("A," Chart 1). After removal of suspended material by filtration the clear filtrate had little effect on the mitoses. When dilute alkali was substituted for the physiological saline solution much less inhibition was seen ("B," Chart 1). No attempt was made to explain the low inhibitory effect of the alkaline extracts, but in subsequent extractions alkali was avoided. The part played by the suspended cells is discussed later in connection with the residue. The efficiency of acid alcohol extraction is illustrated in "C," Chart 1. Unlike the two preceding extracts which were cloudy suspensions, Extract "C" was rendered perfectly clear by the following procedures: fresh kidney pulp was mixed with 3 volumes of 0.2 per cent solution of HCl in 60 per cent alcohol and allowed to stand for 18 hours. Sufficient alcohol was then added to raise the alcoholic concentration to 86.6 per cent. Separation of the clear extract was obtained by filtration. The routine procedure in preparation of this and subsequent extracts was to evaporate the solvent by fanning, to suspend the residue in distilled water and to adjust the reaction to pH 7.4. Residues of the various solvents employed (acetone, chloroform, ether, alcohol) were tested to determine the inhibiting effects of their residues. Likewise NaCl in excess of that present in any of the extracts was injected and found to be non-inhibitory. In Extract "C" not only was the potency of the extract found to be high but the inhibiting substance was shown to be soluble in 86.6 per cent alcohol. Simple alcoholic extractions were then made and the amount of inhibitor appearing in the extracts was found to increase with the concentration of the alcohol ("D" with an alcoholic concentration of 63.3 per cent, "E" with 86 per cent, and "F" with 95 per cent, Chart 1).

With the data indicating that the inhibiting substance was of a lipoid nature, more effective lipoid extractions were inaugurated in attempts to increase the yield of inhibitor. A single 7 hour extraction of kidney desiccate with chloroform gave only 11 per cent inhibition ("G," Chart 1), but a 6 hour extraction with warm chloroform in a Soxhlet extractor yielded a product with a potency of 31 per cent ("H," Chart 1). Following an initial extraction of kidney with acetone, Soxhlet extraction with anhydrous ether for 22 hours gave 17 per cent inhibition ("I," Chart 1). Acid alcohol extracts were almost completely dissolved by chloroform. In one in-

stance 2.03 gm. was removed from 2.18 gm. acid alcohol extract by 7 hours Soxhlet extraction with anhydrous chloroform.

Many extractions have been made for the purpose of increasing the yield of inhibitor per gram of fresh kidney. Maclean¹⁴ pointed out the relative inefficiency of ether or chloroform alone for the removal of lipid and called attention to the better yields obtained by ether (or chloroform) extraction followed by alcohol. After dehydration of fresh kidney pulp with 10 volumes of acetone, a combined extraction with chloroform and alcohol gave a product causing a 40 per cent inhibition ("J," Chart 1). Maclean and Maclean¹⁵ now recommend 4 to 6 extractions with cold absolute alcohol. By this method we obtained no greater degree of inhibition (36 per cent). Jowett and Lawson¹⁶ have recently described a method for the quantitative extraction of lipoids by subjecting the powdered material to three 1 hour extractions with the hot absolute alcohol. We first dehydrated by covering the macerated tissue with absolute alcohol and then gave the material 6 consecutive 1 hour extractions with hot absolute alcohol. The seven fractions were combined and tested. The average inhibition was increased to 68 per cent ("K," Chart 1), which is the best result so far obtained. This is the mean of an 18 day and a 20 day litter of rats. The details of the 20 day litter are given in Table I. It is seen that effective inhibition was accomplished by as small an amount of lipid as that contained in 0.5 gm. of fresh kidney.

TYPE OF LIPOID RESPONSIBLE FOR THE INHIBITORY EFFECT

Although no systematic attempt has been made to fractionate the constituents of the lipid extract, several of the experiments point to the lecithins as the active agent. First the strength of the inhibitor was found to increase in proportion to the efficiency of the lipid extraction. The extracted desiccated material dissolved readily in either anhydrous ether or anhydrous chloroform. When acetone was used as a dehydrating agent it removed some of the inhibitor and it contained not only cholesterol and neutral fat but also phospholipids. In one instance the phosphorus content of the acetone fraction was 1.73 per cent. Determinations of N and P in the usual ether-alcohol extract of kidney by the Maclean¹⁵ method showed an N of 3.12 per cent and a P of 2.79 per cent. Pure cho-

lesterol (Kohlbaum) in a dose of 120 mg. was devoid of inhibiting property while lecithin (practical, Eastman) in a dose of 117 mg. produced 56 per cent inhibition, which compared favorably with the inhibition produced by the lipid kidney extract. A pure lecithin * which had stood in a desiccator for more than 12 years was toxic and lethal to the rat in a dose of 180 mg. and a 11 mg. dose gave a 70 per cent inhibition. Hydrolysis had certainly taken place in

TABLE I

*Experiment 49**Injection of Kidney Lipoid Extract (Jowett and Lawson Procedure)*

No. of rat	Age	Weight at injection	Dosage	Dosage equivalent of fresh kidney	Weight at autopsy	Average No. mitoses per section of kidney
	<i>days</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	
1	20	29.4	0.150*	0.74	32.4	13.5
2	20	31.4	0.030*	0.15	34.1	27.5
3	20	30.4	0.210*	1.04	31.9	16.0
4	20	29.0	0.267*	1.39	29.5	4.5
5	20	32.8	0.074*	0.39	33.2	10.0
6	20	29.0	0.175†	3.01	28.4	2.5
7	20	30.5	0.088†	1.50	31.7	5.5
8	20	32.3	0.029†	0.50	34.6	7.5
9	20	31.3	control		35.4	45.5
10	20	28.4	control		31.3	25.0

* Residue after removal of lipid.

† Lipoid extract.

this preparation and the observed toxicity suggested the testing of choline in the form of the choline hydrochloride. This hydrolytic derivative of the lecithins in a 11 mg. dose gave an inhibition of 43 per cent.

DISTRIBUTION OF THE INHIBITORY AGENT AND ITS LACK OF SPECIFICITY

A limited investigation of the lipid inhibitor content of the different organs was made. Chloroform extraction of rat heart muscle was followed by extraction with alcohol, and the two fractions tested separately on rat kidney. The former gave an inhibition of 22.6 per cent and the latter 24 per cent. Fresh rat liver dehydrated

* Prepared from Brewer's yeast by Dr. Wm. C. Austin. (See University of Chicago Abstracts of Thesis, Science Series, 2, 337.)

with acetone was given four successive extractions with absolute alcohol for a total of 30 hours and the combined extracts tested on rat kidney. The percentage of inhibition was 29.6 per cent.

A number of determinations of the effect of kidney lipoids upon the proliferative activity of the rat liver were made. To do this an 8 mm. disk of liver for paraffin embedding was cut from the formalin-fixed liver and from this paraffin sections were made for staining in

TABLE II

*Experiment 39**Effect of Lipoid Extract of Kidney on the Mitotic Rate of the Liver*

No. of rat	Age	Weight at injection	Dosage	Dosage equivalent of fresh kidney	Weight at autopsy	Average No. of mitoses	
						Kidney	Liver
	<i>days</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>		
1	21	43.6	0.096*	3.89	42.4	14.5	17.0
2	21	42.1	0.024*	0.97	44.0	30.5	13.0
3	21	43.9	0.096†	0.58	39.3	14.5	3.0
4	21	40.9	0.024†	0.15	41.0	10.0	6.0
5	21	46.1	control		49.2	38.5	90.0
6	21	46.8	control		49.6	52.5	23.0

* Combined ether-alcohol extract.

† Residue after removal of lipoids.

hematoxylin and eosin. All mitoses in the hepatic cells were enumerated in several sections. Although there was a wider variation in the mitotic counts made on the livers of the control animals than was noted in the kidneys, conclusive evidence of the inhibitory effect of the kidney lipoids upon liver proliferation was found in the single experiments and in the average for all the experiments. In Table II details of an injected litter are tabulated.

INHIBITORY ACTIVITY OF THE KIDNEY RESIDUES

When separate extractions permitted it, the various individual lipid fractions were tested and in all experiments the inhibitory effects of the residues were determined. Regardless of the method of extraction the final residue remained inhibitory. Doubts exist in regard to the cause of this inhibition. In a majority of the experiments the percentage of inhibition shown by sediments was between 50 and 70. These residues, of course, contained bulky insoluble fiber which was present undissolved in the peritoneal cavity

at the time of autopsy. The fiber alone caused an inflammatory reaction and it was not always free from bacteria.¹⁷ The insolubility of parts of extracts "A" and "B," Chart 1, may account for some of the inhibition that was obtained by these suspensions. Intra-peritoneal injections of physiological saline solution and of low concentrations of certain other salts do not inhibit. This was well brought out in numerous tests made by us of extracts fractionated by isoelectric precipitation.

DISCUSSION AND SUMMARY

The literature dealing with the effect of lipoids on growth is contradictory. Danilewsky¹⁸ found that tadpoles with lecithin added to the water in which they were kept increased in weight faster than control ones kept in ordinary water, but Goldfarb¹⁹ found no evidence of weight increase brought about by lecithin administration. Robertson²⁰ in mice fed with lecithin observed a decrease in body weight. Our rats were always weighed at the time of injection and at the time of autopsy but longer observations were not made. At the 24 hour period the increase in weight often was somewhat less than that of the litter-mates (Table I). Our experiments show that the lipid extracts of the kidney, myocardium and liver exert an immediate antigrowth effect on the kidneys and also on the liver. As shown in Table II, the larger doses almost eliminate mitotic division. The evidence now at hand points to the phospholipids as the inhibiting agent. Previously, one²¹ of us found that a trichloroacetic acid precipitate of an acid alcohol extract of kidney which was rendered lipid-free by chloroform extraction inhibited mitotic proliferation of the kidney, but the amount of this substance present in kidneys is very small since the inhibiting dose was equivalent to 20 gm. or more of fresh kidney. In unpublished experiments it was found that this non-lipoid substance could be obtained equally well from the heart muscle and that it also inhibited liver proliferation.

An observation on the removal of the inhibiting agent by changes in the hydrogen ion concentration of aqueous solutions was made many times and may be of significance in explaining the relation between the lipid and protein fractions of the extracts. When the pH of acid aqueous extracts was brought to 8-8.3 by the addition

of alkali the clear supernatant liquid was no longer inhibitory, and the full inhibiting effect was present in the precipitate. We were of the opinion that the lipoids were thrown out of solution by the iso-electric precipitation of the protein. Sittenfield, Johnson and Jobling,²² in a study of Rous sarcoma, held the view that the inhibiting agent present in filtrates was "apparently combined with the globulin fraction."

Since a technic such as that used by us focuses attention directly on the karyokinetic changes, our interest in the nuclear division was continuously aroused. In the preceding paragraph the presence in the kidney of a non-lipoid inhibiting agent was mentioned, but the amount in terms of inhibition per gram of fresh kidney is as small as 1/40 of that exerted by the extractable lipid. Also it is clear that certain of the hormones inhibit proliferation of the cells that produce them. It is enticing to look upon the inhibition produced by excess insulin, thyroxin, or parathyroid extract as a display of automatic self regulation of growth and metabolic activity. But because these substances exert such a profound influence on general metabolism, the view is at present speculative. With the phospholipids forming an integral and necessary part of the various cells it may be presumed that under our experimental conditions they enter the cells from the blood stream, which carries an excess brought about by absorption from the peritoneal cavity of the injected lipid. The way in which the excess lipid arrests the initiation of new mitoses in the cells of the renal tubules is not clearly indicated at this time.

From the experiments presented the conclusions are drawn: (1) that the lipoids of organs exert an antigrowth effect on the kidney and liver of young rats into which they are injected; (2) that the inhibiting effect exerted by the lipoids is a potent one, since the quantity contained in 0.5 gm. of fresh kidney is active; (3) that the inhibiting substance is probably in the phospholipid fraction; (4) that it is not specific and is not limited in its action to one or more organs; and (5) that it is widely distributed in the body.

An explanation of the function of lipoids in the regulation of cell growth must await investigation of other factors concerned in nuclear division.

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CONGENITAL MEGACOLON *

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The object of this paper is to report a case of *megacolon congenitum*, which in its principal pathogenetic features corresponds exactly with Hirschsprung's classical description of the disease. The case is unusual on account of the physical proportions of the patient, the long duration of the symptoms, and the definite mechanical relation of the lesion to the patient's death.

To present a comprehensive review of the vast literature since 1886 in a single report of this nature would be difficult. Briefly, however, one should bear in mind three distinct classifications of the disease etiology which have grown up in the last half century. There are still numerous reports of the clinical histories and autopsy findings each year in support of Hirschsprung's pioneer theory that the intestinal dilatation and hypertrophy are of a prenatal, developmental nature which, in its genuine form, is never found to arise from an obstruction of a tangible type or from a postnatal lesion. Opposed to Hirschsprung's¹ original theory that the persistent constipation which commences shortly after birth is a consequence of congenital dilatation and hypertrophy of the colon is Lehmann's² contention that the hypertrophy and dilatation must necessarily follow the chronic constipation. This writer, favoring the second theory that an organic obstruction, whether in the form of anal atresia, valvular closure, tumor or kinking of the distal gut is always present, contends that the hypertrophy is of the nature of a "work-hypertrophy" and cannot antedate the obstructive constipation. Aschoff,³ too, prefers the concept of "work-hypertrophy" to explain the pathogenesis of the disease. Actually, in the findings of a number of authors, the degree of hypertrophy in the intestinal wall decreases from the sigmoid colon to the ileocecal valve, in striking analogy to the pyloric hypertrophy which decreases in degree from the *canalis egestorius* to the cardia.

* Received for publication September 11, 1934.

In recent years a third viewpoint as to the etiology of megacolon has been brought forward, a viewpoint that is entirely in keeping with the developing trend toward the neurogenic etiology of disease in all departments of medicine. Ogawa⁴ suggests a relative insufficiency of the intestinal plexuses with subsequent stasis of fecal material, dilatation, and also secondary hypertrophy of the muscle layers. Cameron⁵ has described absence of the cells of Auerbach's plexus. Dott⁶ and Adamson and Aird⁷ have performed sympathectomies for the successful treatment of megacolon, and the experimental parasympathectomies of the latter authors, with the production of megacolon in cats by affording a preponderant influence of the pelvic sympathetic nervous system, are interesting. These writers feel that an indisputable argument in favor of a neurogenic origin of the disease is the occasional association with it of a dilated urinary bladder, the only feature that bladder and lower colon share in common being their nerve supply. Neugebauer,⁸ who also regards the finding of a dilated urinary bladder as of more than incidental importance, seeks an explanation in the development of this organ from the allantois, which in turn originates from the anterior wall of the end gut.

The ingenuity often expressed in neurogenic speculation may be recognized in Knittel's⁹ theory of the pathogenesis of megacolon. He associates the disease with a general vasoneurotic condition and regards it as a vegetative dysergia, a diathesis similar to that which is frequently held responsible for gastric ulcer. The microscopic capillary findings here, he holds, are generally strongly positive.

Finney,¹⁰ in his monograph on the subject, enumerates nine different hypotheses suggested by a multitude of workers in their endeavor to explain the etiology of the affection. They are all more or less closely related to the three larger divisions which have been mentioned above.

REPORT OF CASE

Clinical History: The clinical history of the case, which came to autopsy on May 30, 1934, was obtained from the Department of Health of Yale College, and from the patient's own physician. From early infancy the patient had been afflicted with a markedly distended abdomen which at all times was in extreme disproportion to his general physique. The usual history of infrequent bowel movements, separated by weeks of constipation and often elicited only by cathartic or enema, is given. Nevertheless, the patient reached a height of 6 feet, was graduated from college, and finally became so accustomed to the

difficulties associated with his condition that he came to regard himself as enjoying relatively good health. The remarkable tolerance of this affection on the part of adults has been demonstrated many times. A young man, with symptoms dating from early childhood, is described by Federn¹¹ as having served in a soldier's capacity. Another patient was quite able to participate in strenuous sport.¹²

Our patient, at the time of death, was a student of drama and was considered to be of more than average ability. It is said that he appeared to carry himself almost arrogantly, probably because of the expansion of the lower part of the thorax and the lordosis, both of which usually accompany the condition.

The extent of the condition present in October, 1933, 9 months prior to the patient's death, may be construed from the routine roentgenogram of the chest made by the college Department of Health at this time (Fig. 1). The diaphragm, particularly on the left, is eventrated, the heart is displaced to the right and beneath the dense band of shadow which represents the left diaphragm is the gas-filled contour of what is probably a distended loop of the large intestine. At this examination the patient complained only of chronic constipation, was strongly disinclined to follow treatment of any nature and was lost track of during the following months.

On the morning of May 30, 1934, he was discovered in a sitting posture and gasping for breath by a classmate who had seen him perfectly well on the previous evening. He complained that he could not breathe in a prone position and had, therefore, not slept that night. A physician was summoned, but the patient died before his arrival.

AUTOPSY REPORT

The lips, perioral skin, and the nailbeds of all the fingers and toes were tinged a light blue. The abdomen was enormously but symmetrically distended. It not only bulged anteriorly, to the complete obliteration of the umbilical depression, but the flanks and the soft tissues of the lumbar region on both sides of the vertebral column were also ballooned out. The percussion note was alternately tympanic and dull. Numerous old striae marked the tense skin on either side of the midline. The chest was likewise definitely expanded in its lower portion, the intercostal spaces being widened to 4 and 5 cm. The circumference of the body at the umbilical level measured 125 cm. and the greatest abdominal circumference, about 3 cm. above this point, was found to be 135 cm.

The picture which was presented to view with the exposure of the peritoneal cavity and the removal of the chest plate was accurately reproduced by our artist during the autopsy (Fig. 2). It may be seen that the coils of small intestine, which appeared only slightly distended, were found at the extreme left of the abdominal cavity, in a position normally occupied by the descending colon. A greatly

distended portion of large intestine, identified as the ascending colon, held a central position in the cavity and extended high up to the level of the third rib, at which point the first part of the transverse colon was seen to have raised the diaphragm still higher to a level almost parallel with the left clavicle. The transverse colon came down between the small intestine on the left, and the descending colon assumed a position anterior and to the right of the ascending colon. The descending colon, at a point approximately corresponding to its juncture with the sigmoid, had elevated the right dome of the diaphragm to the level of the third rib. The sigmoid, in turn, lay concealed behind the ascending colon. The dilatation included the rectum as well. The glistening serosal surface of the whole large intestine was marked by the broad branchings of the mesenteric veins. There was no trace of haustra, tænia, or appendices epiploicae. The wall of the entire gut from the ileocecal valve to the internal rectal sphincter had a curious leathery feel. This same length of intestine, stretched out after removal from the peritoneal cavity, measured 250 cm. Some idea of the degree of dilatation may be had from the dimensions which were taken *in situ*. The ascending portion of the colon had a circumference of 35 cm. The circumference of the descending portion was even greater, measuring 56 cm.

One of the largest megacolons reported in the literature, from Formad's¹³ 23 year old patient who was termed a "balloon-man" during life, and compared with an "ox-gut" at autopsy, reached a circumference of 33 to 44 cm. Several examples of megacolon, which appear, however, to have involved little more than the sigmoid and in no instance have included the entire large intestine, have shown even greater dimensions. Vulpian¹⁴ found the circumference of the most enormous sigmoid dilatation to be 70 cm. Figures of 67 cm. and 55 cm. were found by Walker and Griffiths,¹⁵ Banks,¹⁶ and Hoffmann.¹⁷ In these cases the relatively rare example of a colon dilated in all its parts was not present, and hence the 10 liter capacity of the colon in Hoffmann's case is not striking.

After enormous quantities of pasty, foul-smelling, gray-brown fecal material had been removed from our specimen the lumen was filled for the purpose of fixation with 30 liters of 10 per cent formalin. To rule out any possible increase in capacity produced by stretching during this procedure, a series of readings of the circumference

of the gut prior to its removal were taken. These, when calculated with the known length of 250 cm., yielded a result that corresponded closely to the established 30 liters. Several days later the specimen was sectioned and its mucosal surface examined for ulceration or any form of mechanical obstruction. None was seen. The transverse folds appeared ironed out, velvety and entirely intact. A notable difference in degree of hypertrophy, with gradual decrease from the rectum to the ileocecal valve, was manifest grossly in the intestinal wall. Microscopic preparations showed the hypertrophy to involve the muscular coats, including the muscularis mucosae, with a particularly striking increase in breadth of the circular layer. No increase in the fibrous connective tissue between the muscle fibers, as seen by Concetti,¹⁸ could be determined. There was some small round cell infiltration of the submucosa. No abnormalities of number or structure of Auerbach's plexuses were noted.

From the photograph of the black and white drawing (Fig. 2) it may be seen that with the bulk of the liver rotated posteriorly, the anterior aspect of this organ lay parallel with and was compressed against the right diaphragm, permitting only the inferior margin and a small portion of the posterior aspect, including the gall-bladder, to anterior view. The inferior margin of the right lobe of the liver was thin, elongated and concave on its posterior surface, evidently the consequence of chronic intestinal pressure from below. Both lungs were similarly affected topographically. They likewise had been rotated posteriorly. The lower margin of the right lung was at a level with the third rib. Only a small portion of the inferior margin of the left lung was visible, and this protruded slightly below the left clavicle. With the exception, however, of a deep purple and dense area of atelectasis on the upper lateral aspect of the right lower lobe, which measured but a few cm. in diameter, the pulmonary parenchyma was quite crepitant. The elevation of the heart to the upper mediastinum, and its marked displacement to the right were striking. The acute cardiac compression, which produced a kind of tamponade to the normal ventricular dilatation and contraction, together with the extreme passive congestion of the viscera, especially marked in the microscopic preparations of the liver, give us a picture of the mechanism of death which overtook our patient.

DISCUSSION

The manner of the patient's death, reconstructed from the autopsy findings, as well as from the brief account of his extreme respiratory distress during his last few moments of life, is perhaps most worthy of our consideration.

From a table of 17 cases selected by Concetti,¹⁸ in which the cause of death was definitely known, 10 were recorded as resulting from acute colitis. Marked cachexia and perforation of the intestine complicated 2 of these. Of the remaining 7 cases, death resulted in 4 from cachexia alone, 2 were postoperative and 1 resulted from pneumonia. The colitis in the majority of the cases cited was accompanied, as established at autopsy, by a widespread ulceration of the dilated portion of the gut. Finney,¹⁰ who in 1908 had reviewed all the literature on the subject up to that time, stated that heart failure, as well as peritonitis, the result of perforation and diseases of the respiratory organs, particularly bronchitis and bronchopneumonia, were the most common of the intercurrent affections leading to death.

The clinician is frequently faced with significant compression phenomena, such as in our own case. The abdominal distention compresses the thoracic organs and the posterolateral margins of the lungs become atelectatic.^{11,19} The diaphragm in many cases is lost to the respiratory mechanism; breathing becomes thoracic in type and very difficult. Auxiliary respiration with deep retraction at the jugulum and occasionally of the lower intercostal spaces have been observed.²⁰ Barth²¹ described a patient in whom the pulmonary percussion note reached only to the second rib on the left and to the fourth rib on the right. In the same patient the apical impulse was felt in the second interspace. In a 60 year old man, 1 of the 5 cases reported by Wilkie,²² the upward displacement of the liver and the abnormal height of the cardiac dullness were established clinically. Among the symptoms several acute attacks of respiratory distress were noted. This patient made a complete recovery following a preliminary colostomy and subsequent resection of the dilated portion of the bowel.

Definite impairment of cardiac function has been recorded. Perthes²³ noted a periodic bradycardia which disappeared promptly upon the elimination of large quantities of fecal material. Other

authors have found the pulse rapid and feeble.²⁴ It is believed that the cardiac weakness is not only a result of compression of the heart and lungs,²⁵ but that it is also due to the chronic resorption of the products of intestinal putrefaction.¹⁷ The pathologist has been able to find practically negligible evidence of the latter.

SUMMARY

In this report of a *megacolon congenitum* in a 22 year old male stress has been laid upon the magnitude of the intestinal dilatation and its almost exclusive rôle in precipitating the death of the patient. The mechanism of death has been considered in some detail and has been ascribed to cardiac and pulmonary compression, which resulted in the clinical phenomena of acute congestion, strikingly present also at autopsy.

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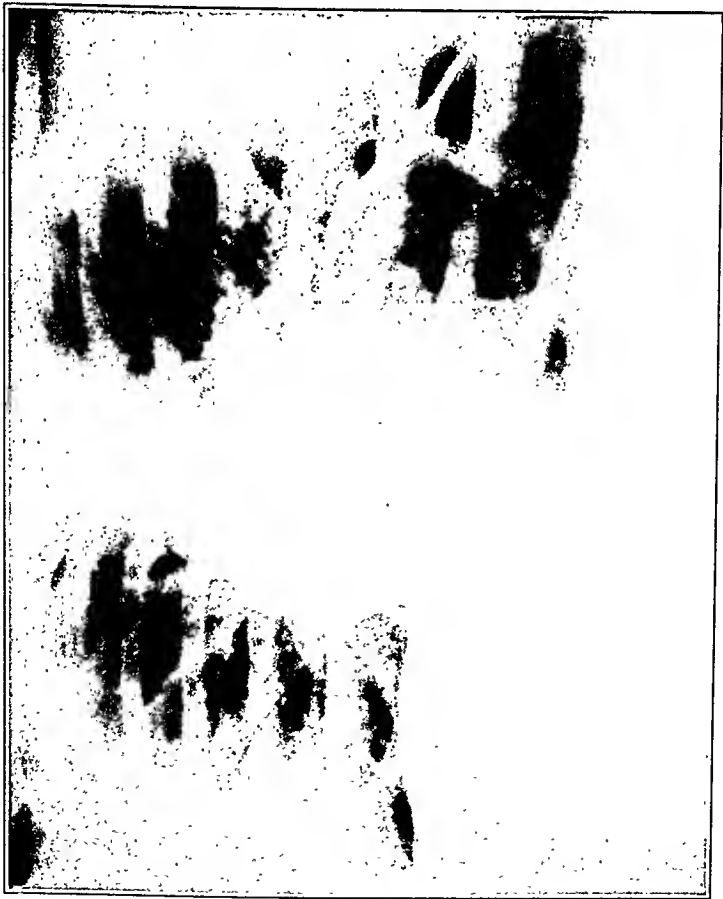
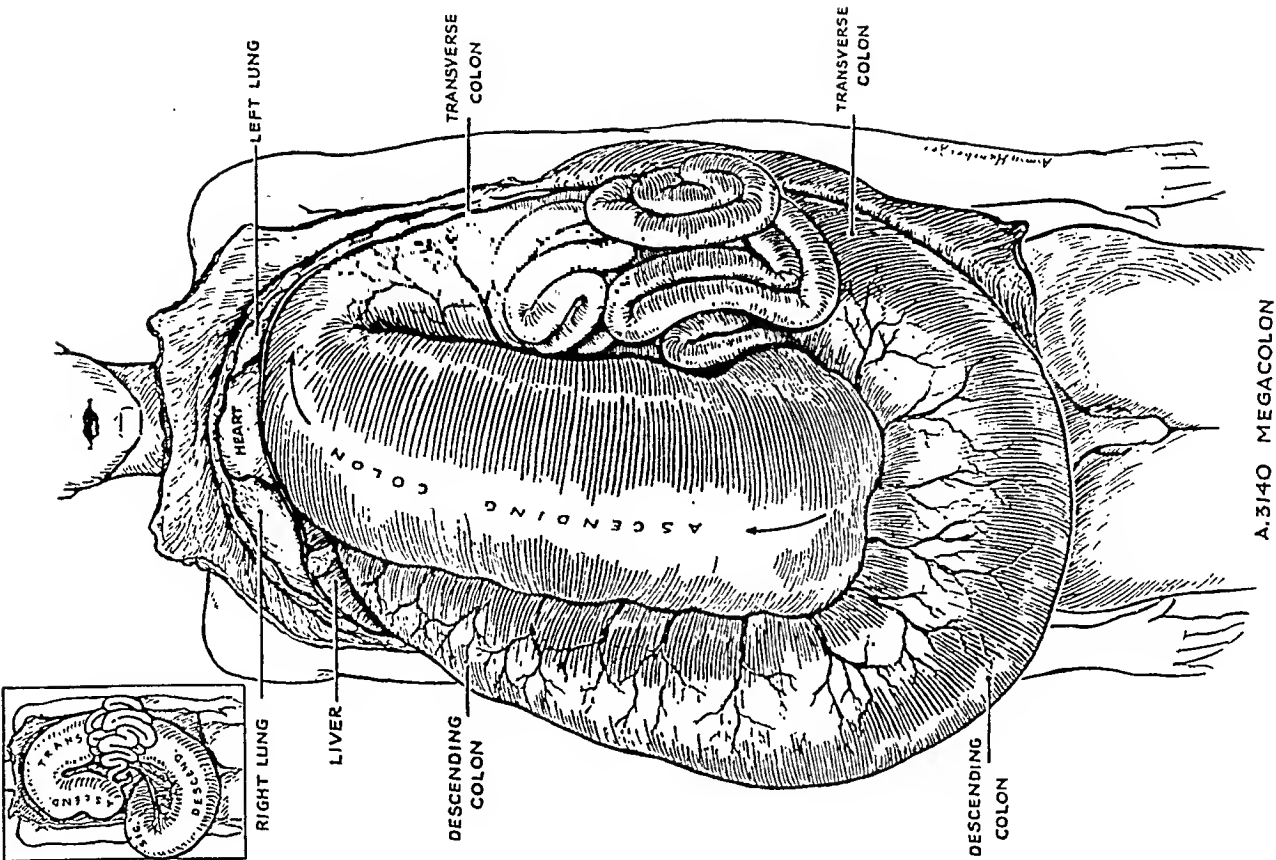
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DESCRIPTION OF PLATE

PLATE 55

FIG. 1. Photograph of roentgenogram of chest showing eventration of left dome of diaphragm, caused by enormously distended loop of large intestine.

FIG. 2. Photograph of black and white drawing of megacolon at autopsy, showing the magnitude of the colonic distention with the upward displacement of liver and thoracic viscera.



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THE AMERICAN JOURNAL OF PATHOLOGY

VOLUME XI

MAY, 1935

NUMBER 3

CYSTIC DISEASE OF THE KIDNEYS *

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Small macroscopic cysts are found in a majority of adult kidneys and in a fair percentage of the kidneys of infants, and if thorough microscopic examinations are made the percentage with cysts is still greater. In a large postmortem service one finds all transitions from typical clinical polycystic kidneys, through subclinical polycystic kidneys to solitary cysts. The structural alterations vary with the number and size of the cysts. Clinical examples of polycystic kidney disease are, however, easily distinguished from clinical solitary cysts. A convenient anatomical classification of renal cysts is as follows:

- I. Cystic disease (typical polycystic kidneys)
 - (A) Bilateral cystic disease
 - (1) clinical $\left\{ \begin{array}{l} \text{large kidneys} \\ \text{normal sized kidneys} \\ \text{hypoplastic kidneys} \end{array} \right.$
 - (2) subclinical
 - (B) Unilateral cystic disease
 - (1) large kidney
 - (2) hypoplastic kidney
 - (3) partial cystic degeneration
- II. Large solitary cysts
- III. Multiple small cysts associated with contracted kidneys

* Received for publication December 17, 1934.

CYSTIC DISEASE

This paper deals only with typical polycystic kidneys. The clinical group includes all those with severe degeneration of the kidneys, and in the adults there were always symptoms referable to the kidneys. The subclinical group includes enlarged kidneys filled with cysts but containing sufficient healthy parenchyma to maintain normal renal function. The lower limit of this group is arbitrary, but no case was included unless the cysts caused a definite enlargement of the kidney or replaced a large proportion of its parenchyma.

Frequency: The reported incidence of polycystic kidneys in post-mortem examinations is shown in Table I. The greater frequency

TABLE I
Frequency of Polycystic Kidneys

Author	No. of polycystic kidneys	No. of postmortems	Ratio
Naumann	16	10,000	1:625
Preitz	16	10,000	1:625
Ward	40	14,000	1:350
Sokoloff	192	50,000	1:260
Watson and Cunningham ...	10	2,429	1:243
Bugbee and Wollstein	13	4,903 (children)	1:377
Wakely	3	3,521	1:1173
Braasch and Schacht	9	9,171	1:1019
Bell	44	22,393	1:509

in some statistics is no doubt due in part to the inclusion of kidneys with only a few cysts. Thus Bugbee and Wollstein include in their group several cases which would be excluded by the more rigid criteria that we have employed, since there were only a few cysts. In this table unilateral polycystic kidneys are included as well as kidneys in which the replacement of parenchyma was not extensive enough to produce clinical symptoms. Of our 44 cases 4 were unilateral and 7 were subclinical; only 33 were advanced cases of bilateral polycystic kidneys.

The frequency in postmortem statistics is also influenced by the number of stillbirths and young infants that are included in the series. In 1819 stillbirths we found 8 examples of polycystic kidneys, and there were 6 cases in infants under 6 months of age. In 17,884

postmortems on individuals over 1 year of age there were 30 examples of polycystic kidneys, 1:596. There were 21 clinical cases in this group, 1:851. Braasch and Schacht found 1 clinical case in each 3523 admissions to the Mayo Clinic.

Unilateral Polycystic Kidney: Cases occur in which one kidney is normal while the other is indistinguishable from the typical bilateral type of the disease. The unilateral cystic kidney may be hypoplastic. Small kidneys measuring from 2 to 6 cm. in length have been reported by Schaefer, Herxheimer, Baumann (2 cases), and Rosenow. The very small kidneys composed of a few cysts are best classified as unilateral hypoplasia. Four cases of this type have come under our observation but have not been included as polycystic kidneys.

Sieber stated that 9 of 150 collected cases were unilateral, but the size of the cystic kidneys was not given. Wakely reported a unilateral cystic kidney, measuring 7 by 5 inches, in a child 20 months old.

Four of our 44 cases were large, unilateral polycystic kidneys, the respective weights being given in Table IV. These kidneys differ in no way from bilateral cystic kidneys. If the four hypoplastic cystic kidneys mentioned above be included, 8 of 48 cases are unilateral. Clinical reports of unilateral cystic kidneys are unreliable since they are usually examples of bilateral involvement with one kidney in a more advanced stage of the disease than the other.

Age: In the literature it is commonly stated that polycystic kidneys occur at two periods, *viz.* early infancy and adult life, and that there is a gap between these ages in which few or no cases occur. In cases collected from a large autopsy service this gap appears (Table II). In our records there is only 1 case between the ages of 4 months and 25 years, and this was unilateral. In clinical experience there are few cases before the third decade. A search of the literature, however, shows that a number of cases in the first and second decades have been recorded. Sieber, in 1901, collected 32 cases between the newborn period and the age of 20 years. In Table II the age distribution of 202 cases collected from the literature is shown. This is only a small fraction of the total cases reported, but perhaps the group is sufficiently large to show the general age distribution. No doubt the number of stillbirths is much too small in proportion to the other groups since these cases are seldom published. In our postmortems nearly one-third of the cases were in the newborn group.

The age recorded usually represents the time when the disease was first recognized either clinically or at postmortem, but in many instances the actual time of onset, as indicated by the symptoms, was many years before the diagnosis was established. Several writers, in studying families with hereditary polycystic kidney disease, have recognized cases in which no subjective symptoms had developed. When these facts are taken into consideration the newborn and the adult groups are brought closer together. There is no longer any

TABLE II

Age Distribution of Author's Cases and Cases Collected from the Literature

Age	Author's cases		Cases collected from literature
	Polycystic kidneys	No. of autopsies	
Stillborn	9 (1 unilateral)	1819	28
0 to 1 mo.	4	2672	6
1 to 6 mo.	1		4
6 mo. to 1 yr.	1 (unilateral)		0
1 to 5 yrs.	0	1147	6
5 to 10 yrs.	0		1
10 to 20 yrs.	0		7
20 to 30 yrs.	2	1759	20
30 to 40 yrs.	4	2445	28
40 to 50 yrs.	8 (1 unilateral)	2944	57
50 to 60 yrs.	6	3146	33
60 to 70 yrs.	6 (1 unilateral)	3077	6
70 to 80 yrs.	0	1780	4
80 to 90 yrs.	2	448	2
Adult (age ?)	1		
Total	44	22,112	202

doubt that the disease is always congenital and that it is the same disease in adults as in infants.

Apparently in about one-third of the cases the individuals are either born dead or die within the first month. Of those that survive over a month only a few die before the age of 20 years and the maximum death rate occurs in the fifth decade. If the disease is the same in adults as in infants, and death is due chiefly to destruction of renal tissue by cysts, why are there so few deaths between early infancy and the third decade? Why do the majority first develop symptoms after the age of 40 years? It has been suggested that in young persons the renal parenchyma between the

cysts undergoes hypertrophy to compensate for the tissue destroyed by progressive expansion of the cysts, and that in adults this compensatory hypertrophy no longer occurs. It is well known that the adult kidney does not hypertrophy as readily as that of a child. Another factor of importance is the vascular disease which develops slowly and reduces the blood supply of the parenchyma. This will be discussed more fully in subsequent paragraphs.

Sex: Large statistics indicate that there is no difference in the incidence in males and females. Braasch and Schacht had 98 females and 95 males in their group of 193 patients. In our postmortem series there were 20 males and 23 females. In the cases collected from the literature there were 96 males and 96 females.

Clinical Types: (A) *Surgical Type:* Often the initial symptoms are those of unilateral renal disease. There may be pain in the region of one kidney or an attack of hematuria. When the opposite kidney is not palpable an incorrect diagnosis of neoplasm may be made, and when the kidney is not notably enlarged tuberculosis and calculus must be considered. Gross hematuria occurs in about one-third of all patients (Braasch and Schacht). The pain is often caused by ureteral spasm during the passage of blood clots. Occasionally one or more cysts become infected, giving rise to pyuria and other symptoms suggestive of pyelonephritis. When the function of the two kidneys is studied separately it is sometimes found that blood and pus come only from one kidney and that it excretes little or no indigo-carmin, while the function of the opposite kidney is good. Under these circumstances it may be justifiable to remove the non-functioning kidney. A great many writers are opposed to nephrectomy, particularly because of the associated high mortality, but there are many published reports in which a long period of relief followed this operation. Blatt's patient was living 15 years after nephrectomy and the other kidney was not palpably enlarged until 12 years after the operation. Rumpel's patient was living and well 12 years after nephrectomy. The affected kidney excreted no indigo-carmin. In a case reported by Blum pain developed in the region of the left kidney at the age of 15 years. The pain continued and the patient had frequent attacks of hematuria until the age of 21 years, when the left kidney was removed because of very low function while that of the right kidney was normal. Twelve years later, at the age of 33 years, the right kidney first began to enlarge and became painful. The

patient was living at the age of 38 years, 17 years after nephrectomy. Walters and Braasch recommend nephrectomy when one kidney shows infection with a marked reduction of function. In thirty-one nephrectomies they had only one postoperative death, and 18 of their patients were living from 1 to 19 years after the operation.

Calculi are occasionally found in the polycystic kidney and may be largely responsible for the symptoms (Blatt, Cumming, Walters and Braasch, 5 cases). Rarely the cystic kidney contains a malignant neoplasm (Walters and Braasch, 3 cases), and occasionally tuberculosis is found (Uteau).

(B) *Medical Type*: The majority of persons with polycystic kidney disease come to the attention of internists because of symptoms referable to renal insufficiency. The clinical picture is often similar to that of chronic glomerulonephritis. The onset of uremia may be sudden but more often it develops slowly over a period of years.

Albumin is found in the urine from a trace to a large amount in the great majority of cases (180 of 190, Braasch and Schacht). This is probably due to interference with the blood supply of the glomeruli from pressure of cysts and from stretching and compression of the arteries and veins.

Gross hematuria is found in about one-third of the cases from time to time. The hematuria occurs usually in the form of attacks separated by intervals of varying length. Hemorrhage takes place into the cysts from stretching and tearing of vessels in their walls, and since frequently some of the cysts communicate with the pelvis blood escapes into the urine.

The urine is of low specific gravity and concentration tests show diminished powers of concentration. In a few instances polyuria has been reported (Götzl, Shapiro, Veil). In the terminal stages oliguria occurs frequently. The loss of ability to concentrate the urine is similar to the condition in contracted kidneys and is due to reduction of functioning parenchyma.

Edema: This is a rare symptom. Cases with edema were reported by Piersol (legs and abdomen), Shapiro (2 with edema of ankles), Atonna and Morrissey (edema of legs), Fahr (edema of feet), and Strübing and Pugh (marked generalized edema). There was a slight edema of the ankles in 5 of our cases, Nos. 39, 40, 42, 43, 44. The cause of the edema in these instances was not established, but cardiac decompensation is a possible explanation. There has apparently

been little or no study of the serum proteins, but one would not expect to find a protein depletion in the blood since there is relatively little loss of protein in the urine. The usual absence of edema indicates that cardiac decompensation plays an unimportant rôle in this disease.

Blood Pressure: Rosenberg maintains that hypertension seldom results from polycystic kidneys. In an earlier publication, before much clinical data on this point was available, I expressed a similar view based largely on the size of the heart, but since that time I have had a wider experience with this disease and a number of publications have appeared which show clearly that this view is erroneous and that hypertension is usually present in advanced stages of the disease.

In Table III all the cases are listed in which the author gave data on blood pressure or size of the heart, and in Tables IV, VI and VII our own observations are recorded. The blood pressure was recorded in 82 patients, 64 from the literature and 18 from our series. The highest blood pressure obtained is usually recorded. The highest systolic pressure was 145 mm. Hg or more in 48 cases, and was below this level in 30. In Veil's 3 cases and in 1 of ours it is merely noted as high. If we take 150 mm. Hg as the lowest level of hypertension, there are 44 at this level or above and 34 below. Of the 44 with elevated blood pressure, 22 had moderate hypertension (150 to 175 mm. Hg) and 22 had severe hypertension (175 mm. Hg or above). More than one-half of the cases (44 of 78) had a definite hypertension.

This is in agreement with the extensive study by Schacht, 1931, who found a systolic pressure of 145 mm. Hg or more in 61 per cent of 193 patients at the Mayo Clinic. The individual cases are not reported and therefore cannot be included in the table. Ritter and Baehr studied 3 cases where hypertension and uremia were present; no details were given.

Fahr suggested that the cases of polycystic renal disease without hypertension were those in which the parenchyma was not so extensively atrophied. As shown in Tables III and VII, the blood pressure was recorded in 41 cases with satisfactory clinical or anatomical evidence of rather marked renal insufficiency. In 27 of these the systolic blood pressure was 150 mm. Hg or more, while it was below this level in 14. This confirms the observation of Braasch and

TABLE III

Cases Collected from Literature Presenting Data in Polycystic Renal Disease

Author	Date	Age yrs.	Sex	Blood pressure	Renal function	Weight of heart gm.	Weight of kidneys gm.	Comment
Kaufmann	1932	33	F	145/80	Concentration diminished	No hypertrophy	?	Living
Rosenberg	1932	49	F	130/?	?	"	?	
"	"	44	F	145/?	?	"	?	
"	"	45	F	160/?	?	550	?	
"	"	53	M	170/?	?	Marked hypertrophy	?	
Blatt	1927	55	M	?	Concen. test 1010-1011	?	?	
"	"	23	F	110/? (6 yrs.)				
"	"	36	F	155/?	?			Duration 10 yrs. living
"	"	43	M	130/?	?	?	?	
"	"	41	F	165/?	?	?	?	Symptoms of uremia
"	"	?	M	135/?	Concen. test 1000 to 1017	?	?	
Halbertsma ...	1931	10	F	150/110	Concen. test 1010-1019, PSP 35			
"	"	40	M	210/170	Urea N 179, PSP 9			Living Duration 16 yrs.
Götzl	1933	54	M	200/130	NPN 155, 220	?		
Fuller	1929	74	M	170/110			Very large	
"	"	45	M	190/120	Urea 90		Large	Living
"	"	43	F	150/?	Urea 20.8		Large	Living
"	"	42	M	150/115	Urea 39		Large	Living
"	"	40	M	145/100	Urea 45		Large	Living
"	"	40	M	185/120	Urea 40		Large left	Living, 7 yrs. duration
"	"	16	F	100/?	Concen. good		En- larged	Living
"	"	18	M	105/80	Urea 13.6, concen. good		Pal- pable	Living

NPN = non-protein nitrogen. Urea N = urea nitrogen, mg. per 100 cc. of blood. PSP = phenolsulphonephthalein, per cent in 2 hours.

Podgurski	1930	41	M	120/95	?	Enlarged	Very large
"	"	52	M	160/105 195/100 180/105 145/95	Urea 141, 203 Urea 53, NPN 56	550 Marked hypertrophy	Very large Very large Three times normal
"	"	48	M	150/90	Concen. test 1008-1024	Hypertrophy	Very large
"	"	48	F	140/105 (3 yrs.) 135/70 180/75	Concen. test 1001-1010 Urea 68 (3 yrs.), 374 (recent) Concen. test 1008-1010	Hypertrophy Marked hypertrophy	2000 Very large
"	"	43	M	215/? 145/? 161/?	NPN 126	360	2200
Fahr	1929	45	M				
"	"	61	M		?	380	Large
"	"	46	M		?	440	Left
"	"	45	M	140/?	NPN 348	520	950
"	"	55	M	210/?	?	555	520
"	"	85	F	145/?	NPN 146	Not enlarged	Large
Piersol	1928	42	M	130/60	Concen. test 1006-1010	Moderate hypertrophy	?
"	"	64	M	218/165	Urea N 160, creatinin 21		4420
Washburn	1930	47	F	140/90 (1 mo.)	Urea 23.8 (14 mo.)	?	1985
Shapiro	1929	55	F	138/74 (6 yrs.) 180/? (3 yrs.) 160/? (2 mo.) 186/? (1 yr.)	?	?	1575
"	"	29	M			?	?
"	"	36	F	134/80	NPN 42, urea 21	?	?
"	"	10	M	140/82	NPN 78, urea 38		
"	"	42	M	126/82	NPN 69 to 342, creatinin 10.3		
"	"	46	M	140/90 190/120 220/?	NPN 34.2, urea 19.6	?	?
"	"	48	M				
							Living
							Living
							Living

TABLE III (Continued)

Cases Collected from Literature Presenting Data in Polycystic Renal Disease

Author	Date	Age yrs.	Sex	Blood pressure	Renal function	Weight of heart gm.	Weight of kidneys gm.	Comment
Shapiro	1929	37	M	162/110	NPN 375, urea 250	?	8m. Lcft	
"	"	52	M	164/96	NPN 160, urea 54, creatinin 10.4	?	5220	
"	"	28	F	130/90	Urea 32, PSP 60		2717	Living
"	"	42	F	122/84 (7 yrs.)	?			Living
Gottlieb	1925	28	F	120/75	Sp. gr. 1000 to 1018			Living
Litzner	1929	40	M	140/?	Urea 354, concn. test 100.4-1010	?	Large	
"	"			150/?				
Ludowigs	1926	46	M	135/?	Delayed excretion of indigo-carmin	?	?	Living
Grauhan	1926	43	M	105/75	Concn. test 1003-1017, NPN 63			Living
"	"	46	M	170/120	Concn. test 1001-1020, NPN 61			Living
"	"	42	F	160/?	Concn. test 1001-1011, NPN 43			Living
"	"	40	F	120/70	Concn. test 1006-1011, NPN 112			Living
"	"	36	M	240/?	NPN 189			Living
"	1926	44	F	106/55	?		?	
Veil	1914	52	F	Elevated	No retention of nitrogen	?	?	
"	"	42	F	"	PSP 50 in 4 hrs.	?	?	
"	"	46	F	"	PSP 7, NPN 48	?	?	
Greene	1922	3.5	F	108/70		50	17	
"	"			100/65				
Cumming	1928	31	M	168/132	PSP 65	?	?	
"	"	36	M	140/90	NPN 172			
Katz and Mülhe	1924	35	M	153/126	NPN 97 to 60			
"	"	45	F	155/100	NPN 34, concn. test 1009-1011	Slight hypertrophy		Living Death from intracranial hemorrhage
McKinlay	1920	30	M	230/130	?	400	Very large	

Schacht that there is a somewhat higher incidence of hypertension in the more advanced stages of the disease. Several authors in studying individual cases over a period of years have noted a gradual rise of blood pressure.

It may be concluded that a systolic pressure of 150 mm. Hg or higher is found in over half of adults with clinical symptoms, and that the percentage with hypertension is still higher in the group with marked renal insufficiency. However, cases occur in which no hypertension develops. The basis of the hypertension will be discussed in a subsequent paragraph.

Hypertrophy of the Heart: In the literature we have found the weight of the heart recorded in only 8 adults, the average weight being about 470 gm. In 7 cases the heart was described as hypertrophied, and in 4 as not enlarged, no weights being given.

The weight of the hearts in our series is shown in Tables VI and VII. In the subclinical group (Table VI) no heart weighs over 400 gm., and 4 of them weigh less than 325 gm. In the clinical group (Table VII) the weights are as follows: 250 to 350 gm. 7 hearts, 350 to 450 gm. 5 hearts, over 450 gm. 6 hearts. In the clinical group the hearts are definitely larger. It may be concluded that cardiac hypertrophy occurs frequently in polycystic renal disease but that it is not as prominent a feature as one would expect in view of the frequency of hypertension. Perhaps the blood pressure is not so high over a long period of time as in primary hypertension.

Renal Insufficiency: The majority of the symptoms in polycystic renal diseases are due to renal insufficiency. The clinical phenomena in the usual case are such as are found with contracted kidneys. There may be gastro-intestinal symptoms, such as loss of appetite, nausea, vomiting, constipation or diarrhea. Anemia and weakness are common symptoms, and there may be a marked loss of weight. Periods of malaise and headache occur. A definite impairment of renal function is demonstrable in about two-thirds of the patients when they first consult a physician. The functional disturbances are similar to those found in patients with contracted kidneys. There is a decreased elimination of phenolsulphonephthalein, a retention of nitrogenous substances in the blood and a decrease of the ability to concentrate the urine. These tests indicate a decrease of functioning parenchyma in the kidneys. Death is usually due primarily to renal insufficiency, unless one of the kidneys becomes infected.

Cerebral Hemorrhage: Sieber, in his survey of the literature in 1901, found that 10 of 212 patients died of cerebral hemorrhage. Dunger reported the death of a woman, aged 54 years, from a ruptured aneurysm in the corpus callosum. Her daughter died at the age of 26 years from a pontine hemorrhage. Blatt noted death from cerebral hemorrhage in a woman 46 years old. Katz and Mühe found a ruptured aneurysm of the anterior communicating artery in a woman 45 years old, and McKinlay described an intracranial hemorrhage in a male 30 years of age. One of our cases, a male 40 years of age, suffered a hemiplegia at the age of 37 years but died of uremia. The incidence of cerebral hemorrhage is low and it is possible that it is due to an associated arteriosclerosis or primary hypertension independent of the renal disease. The rise of blood pressure caused by cystic kidneys would, however, tend to cause rupture of arteriosclerotic vessels in the brain.

Duration of Symptoms: Braasch and Schacht found that 45 per cent of 193 patients lived less than 4 years after the onset of symptoms. Twenty-five lived more than 10 years, and 9 more than 20 years. Cases of long duration are reported by several authors: Blatt (8 yrs., 10 yrs., 10 yrs., 18 yrs.), Halbertsma (16 yrs.), Wulff (15 to 20 yrs.), Collinson (11 yrs.), Götzl (16 yrs.), Fuller (12 yrs.), Blum (23 yrs.), Atonna and Morrissey (12 yrs.), Niecke (11 yrs.), Cumming (8 yrs., 9 yrs., 16 yrs.).

Retinal Changes: Braasch and Schacht found the fundi negative in 43 per cent, retinal sclerosis without retinitis in 31 per cent, and retinitis with retinal sclerosis in 20 per cent of their patients. There are apparently no other studies of the eyegrounds in the literature dealing with polycystic renal disease, but in the literature of hypertension there are occasional descriptions of a retinitis in this form of renal disease. It is believed that the retinitis results from high blood pressure, since it resembles the retinal changes seen in other forms of hypertension.

Effects of Pregnancy: Pregnancy increases the work of the kidneys and not only intensifies any preëxistent symptoms but may also bring out symptoms in a compensated or latent stage of the disease. Blatt described a patient, 36 years of age, who first developed symptoms during pregnancy. There was marked edema, albuminuria and vomiting. The child was born at 8 months, and for 1 year afterwards there was a general weakness, albuminuria and headache.

The patient then remained well for 5 years, after which the symptoms reappeared. A patient reported by Heinsius developed marked edema, heavy albuminuria, visual disturbances and severe dyspnea during the seventh month of pregnancy. At postmortem abscesses were found in the large cystic kidneys and the liver also was cystic.

Symptoms apparently do not develop during pregnancy unless the renal reserve is low. In Podgurski's case the patient went through thirteen pregnancies and first developed renal symptoms at the age of 73 years. There are many cases of this type in the literature.

The unfavorable effects of pregnancy are the same as those occurring in chronic glomerulonephritis and are due to the same cause, *viz.* a marked decrease of renal parenchyma. Pregnancy puts an additional load upon the kidneys and when the renal reserve is low symptoms of renal insufficiency develop.

Influence of Heredity: It has been known for many years that inheritance plays a rôle in polycystic renal disease. Families with a high incidence of the disease have been reported by Dunger, Paus, Cairns, Fuller and Shapiro. Dunger, 1904, found the disease in 5 children of the same mother. In addition he observed the disease in a mother 54 years old, and in her daughter aged 26 years. Paus, 1914, in the family which he studied found 4 members in the first generation, 2 of whom had cystic kidneys. One of those with cystic kidneys had 14 children, of whom 4 had cystic kidneys; the other with cystic kidneys had 3 children, none with the disease. One of the normal members of the first generation had 4 normal children but a granddaughter had cystic kidneys. Cairns, 1925, noted 10 cases in three generations of a family. Fuller, 1929, described 9 cases in 27 members of a family in four generations. Shapiro, 1929, found the disease in a mother, in 4 of her children, and in 1 of her grandchildren.

In addition to these larger groups a great many writers have called attention to more than 1 case in a family. Beck, 1901, reported 3 sisters with polycystic renal disease. Bunting, 1906, found the disease in 2 newborn children of the same mother. Wobus, 1918, found 4 children of the same mother with polycystic kidneys. Rumpel, 1921, found two families each with 3 cases of cystic kidneys in two generations. Cumming, 1928, found a familial history of the disease in 11 of 31 cases which he studied. Halbertsma, 1931, found

the disease in a man 40 years old and his daughter 10 years of age. Balogh, 1933, reported 3 cases in the first generation of a family and 3 in the second. A great many observations similar to those mentioned above have been published. Maier, 1924, quoted twenty-four writers who had found examples of hereditary polycystic kidney disease, and Bunting also gives a number of references. It is clearly established that heredity plays a remarkable rôle in polycystic renal disease. The disease may be transmitted by either sex and it is probably a dominant character. It is not known whether apparently normal persons who transmit the disease to their offspring have normal kidneys or a latent form of cystic disease.

Renal Rickets: Like other forms of chronic renal disease polycystic kidneys in children may lead to dwarfism or rickets. Greene, 1922, described a severe case of rickets in a girl $3\frac{1}{2}$ years of age. The child was underdeveloped and underweight and had a marked renal insufficiency. The cystic kidneys were small, 10 gm. and 7 gm.

It is probable, however, that Greene's case was not true congenital cystic renal disease, but hypertrophy and cystic dilation of tubules secondary to some form of atrophy or hypoplasia. This topic is discussed more fully in a subsequent paragraph. Mazzeo, 1930, described a rachitic dwarf 10 years of age with renal insufficiency due to cystic kidneys. The height was 88 cm.

Diagnosis: Braasch and Schacht in 193 patients found bilateral palpable kidneys in 151, enlargement of one kidney only in 30, and no enlargement in 12. When both kidneys are enlarged there is usually little difficulty in establishing the diagnosis. There may be local pain or tenderness in one or both kidneys or symptoms due to pressure on the intestines or other structures, but for the most part the symptoms are referable to renal insufficiency and do not differ essentially from those associated with chronic glomerulonephritis. A moderate edema of the lower extremities is occasionally seen, but marked edema is very unusual. Cardiac decompensation seems to be uncommon. The attacks of hematuria are peculiar to this disease.

The cases in which only one kidney is enlarged present some diagnostic difficulties and have been confused frequently with renal tumor or hydronephrosis. When a calculus is demonstrated, as happens occasionally, the nature of the underlying disease is even more difficult to recognize. It is not unusual to find a marked difference in the size of the two kidneys even when both are enlarged. They

may also exhibit striking differences in function. When the disease is demonstrable in only one kidney, it by no means follows that it is not bilateral. In a case reported by Blum the right kidney first showed a demonstrable enlargement 12 years after the left had been removed. In a unilateral enlargement an accurate pyelogram usually leads to the correct diagnosis. Since Adrian and von Lichtenberg pointed out the characteristic shape of the pelvis, as revealed by the pyelogram, the accuracy of the clinical diagnosis has improved markedly. The most convincing pictures are obtained on large kidneys, the typical appearance being elongation of the pelvis and calyces. The calyces may be widened, especially at their tips, or one or more calyces may be flattened or obliterated. The lengthening of the pelvis and calyces is due to the increased size of the kidneys and their distortion is due to the encroachment of cysts upon them. In small kidneys the diagnosis may be uncertain.

When neither kidney is enlarged, as in our Case 43, a diagnosis of chronic glomerulonephritis or hypertensive kidney is usually made and the true nature of the disease is not recognized. In our case there was hypertension, cardiac hypertrophy and renal insufficiency, and no symptom or sign suggesting cystic kidneys. Possibly a pyelogram would have given the correct interpretation.

PATHOLOGY

Polycystic kidneys retain the shape of a normal kidney, the various dimensions being increased proportionally. There may be some displacement caudally because of the increased weight. When very large they fill the lateral retroperitoneal areas, displacing the intestines anteriorly and medially.

The external surfaces are closely set with rounded elevated areas corresponding to the underlying spherical cysts. On section a honeycomb appearance is noted. In advanced cases the cysts are separated only by narrow bands of tissue, and little or no normal parenchyma is to be seen (Fig. 1). Occasionally, even in advanced cases, small islands of parenchyma 1 cm. or more in diameter are found, and in cases in which death is not due to renal insufficiency there may be a large amount of normal parenchyma between the cysts (Fig. 2). When the parenchyma is reduced to a minimum the persistent portions are usually in the subcapsular zone. Usually

both cortex and medulla are filled with cysts and no distinction between these portions can be made, but in rare instances there are no cysts in the medulla (Herxheimer, Case 1). Schaefer described a kidney with a row of cysts at the cortico-medullary junction and none elsewhere. One pole of the kidney may be filled with cysts and the other pole normal (Cases 4 and 20). In the smaller kidneys the cysts may all be small and of uniform size, giving a spongy texture to the tissue; in the usual case, however, there is a rather marked variation in size, an occasional cyst approaching the dimensions of a large solitary cyst. In general there is a direct relation between the size of the kidney and the size of its cysts, and one gets the impres-

TABLE IV
Unilateral Polycystic Kidneys

Case No.	Autopsy No.	Age	Sex	Blood pressure	Weight of heart	Weight of kidneys	
						Right	Left
		<i>yrs.</i>			<i>gm.</i>	<i>gm.</i>	<i>gm.</i>
1	20-260	6 mo.	M	?		73	8.5
2	28-1732	45	F	?	325	500	210
3	30-1811	Sb	F			10	33
4	34-987	65	F	195/110	499	177	666

sion that enlargement of a cystic kidney is due to increase in the size of the cysts rather than to an increase in their number.

Frequently some of the cysts communicate with the calyces. When hematuria occurs it is commonly due to bleeding within a cyst that communicates with the pelvis. These communicating cysts may represent primary outgrowths from the pelvis or their connections may have been established secondarily. The cysts are filled with a watery fluid which is usually clear but sometimes colored brown, black or red from admixture of old or fresh hemorrhage (Davis). Chemical examination reveals a high content of urea, uric acid and creatinin. In Piersol's patient the fluid from the cysts contained: uric acid 20 mg., creatinin 28 mg., and urea nitrogen 300 mg. per 100 cc. Litzner found: urea 321.8 mg., and uric acid 12.6 mg. per 100 cc. In both of these cases there was a marked retention of nitrogen in the blood corresponding roughly to the content of the cystic fluid. Singer and Brams found 45.9 mg. of urea per

100 cc. in the cystic fluid of a newborn infant. Strübing found serum albumin and serum globulin as well as urea in cystic fluid. The

TABLE V

Bilateral Polycystic Kidneys in the Newborn

Case No.	Autopsy No.	Age	Sex	Crown-heel length	Cystic liver	Weight of heart	Weight of kidneys	Arteries	Comment
5	18-258	14 wks.	F	cm. 56	—	gm. ?	gm. 60 65	?	Congenital syphilis
6	20-243	11 da.	F	50	—	?	100 65	Normal	No edema, no note on the urine
7	21-33	30 min.	M	49	—	35	155 160	?	Bilateral hydrocele
8	24-207	Sb	M	47	—	29	67 69	?	
9	26-880	Sb	M	?	+ many cysts	18.4	407 567	Normal	Meningocele, anomaly of tongue
10	27-663	5 da.	M	44	—	20	122 114	Normal	Clinical uremia
11	27-1184	Sb	M	44	—	16	259 275	Normal	Club foot, undescended right testis
12	30-257	Sb	F	37	—	18	300 275	Normal	Encephalocele, club foot, supernumerary toe
13	33-169	17 da.	M	52	—	?	Large	Medial fibrosis	Oliguria. Albumin, pus and blood in the urine
14	34-1703	Sb	?	?	—	?	20 20	Normal	
15	26-983	Sb	F	39	—	?	21 25	?	Anencephaly, craniorachischisis
16	31-1201	Sb	M	32	—	?	24 21.5	?	Filled with small cysts

Sb = stillbirth.

presence of a high urea content in cystic fluid is to be attributed to diffusion from the blood plasma and not to secretion of urine: pericardial fluid also has a high urea content in renal insufficiency. There

are apparently no observations on the urea content of cystic fluid from cases without renal insufficiency.

The unilateral cystic kidneys (Table IV) will be discussed under the newborn and the adult groups.

Cystic Kidneys in the Newborn

In the group of newborn infants there is an enormous variation in the size of the kidneys. In the 12 cases in our series the combined weight of the kidneys varied from 40 gm. to 974 gm. (Table V). The larger kidneys usually have larger cysts but not a greater number than are found in the smaller kidneys. The destruction of parenchyma is often as extensive in small kidneys as in large ones. In estimating the extent of the renal enlargement the crown-heel length of the infant should be considered, since many of them are premature and the relative increase in size of the kidneys is therefore greater than the weight indicates.

The markedly hypoplastic cystic kidneys have not been included in this study since their pathogenesis may be different.

On section the cortex seldom can be distinguished from the medulla and all parts of the organ are filled with cysts. Often there is a defective development of the pelvis and calyces, and some writers have stressed aplasia of the medulla (Staemmler).

Our cases of bilateral polycystic kidneys in the newborn are listed in Table V. A more detailed description will now be given of the 7 cases in which material was available for microscopic study.

CASE 6. On gross examination numerous very small cysts were found which gave a spongy texture to the parenchyma.

Microscopically most of the glomeruli and tubules are found in small groups in the subcapsular zone, but a few are scattered between the cysts. The medulla is composed of large cysts separated by dense connective tissue; there are no collecting tubules. An abundant, rather dense connective tissue is found in the deep part of the cortex. Some of the normal tubules in the subcapsular zone open into the smaller peripheral cysts. A great many cysts in the cortex, and some of those in the medulla contain a glomerulus projecting into the cavity.

CASE 9. Microscopically there are small islands of normal parenchyma in the subcapsular zone. Nearly all of the kidney is composed

of cysts separated by an enormous amount of loose connective tissue. Very few collecting tubules are found. There are a few tubules and glomeruli in the connective tissue between the cysts. Many small cysts in the subcapsular zone contain a glomerulus projecting into the cavity, and a few of these cysts may be traced into dilated tortuous tubules.

CASE 10. On gross examination the kidney showed a spongy texture and no large cysts were visible.

Microscopically the cortex is composed of long, dilated, tortuous tubules connected with glomeruli in the subcapsular zone. Between the dilated tubules are many atrophic glomeruli with a short segment of tubule or without a tubule. There is a great decrease in glomeruli. The medulla is very fibrous and filled with cystic structures. There is no fibrosis of the cortex.

CASE 11. Microscopically the superficial zone of the cortex is intact. In the deeper part of the cortex the tubules become dilated and some of them open into fairly large cysts. The medulla is composed of very large cysts separated by an abundant, loose, areolar tissue which extends into the deeper part of the cortex. There are only a few collecting tubules. None of the cysts contains a glomerulus.

CASE 12. Microscopically there is a thin subcapsular zone of glomeruli and underdeveloped tubules. Cysts fill the medulla and the cortex up to the subcapsular zone. There is a marked reduction in the number of glomeruli. The medulla is composed of dense fibrous tissue suggesting the medullary fibromas found in adults. There are no collecting tubules. Normal tubules may be traced into small cysts which seem to be dilated segments of the tubule.

CASE 13. On microscopic examination no normal areas of parenchyma are found. There are cysts of varying size and duct-like structures separated by dense fibrous tissues. Occasionally a glomerulus with a short tubule is seen. The walls of the arteries and arterioles are thicker than normal. They show a marked medial fibrosis but no intimal thickening. There is no narrowing of their lumens.

CASE 14. The ureter, pelves and calyces are present. The pyramids are not distinct. Cysts are found throughout the cortex and medulla. There are only a few small nests of glomeruli and tubules

between the cysts. The cysts are separated by a fairly dense connective tissue. A few cysts contain glomeruli. The arteries curve around the larger cysts.

CASE 1. The unilateral cystic kidney of an infant 6 months old (Table IV) may be considered with this group. It presented a structure similar to the preceding cases but the parenchyma was not so extensively destroyed. On section a few small islands of fairly normal parenchyma were seen. There was a small ureter but no pelvis or calyces could be found. The cysts were closely set and some reached a diameter of 4 cm.

Microscopically the small islands of cortex are composed of normal tubules and glomeruli. Many of the cysts are tubular structures with a fibromuscular wall which Busse considered evidence of their origin from the pelvis. There are occasional rudimentary tubules between the cysts. The interstitial tissue is abundant and dense, especially in the medulla. No collecting tubules are seen.

Comment

One of the most prominent microscopic features is an enormous increase of interstitial connective tissue. All of the authors comment on this peculiarity, although a few state that it is not always conspicuous. It varies from a loose areolar to a dense fibrous structure and is usually more abundant and dense in the medulla than in the cortex. The superficial part of the cortex under the capsule is often free from this connective tissue increase and it is in this region that islands of fairly normal parenchyma may be found. In the medulla the connective tissue replaces nearly all of the collecting tubules and it may show dense rounded masses that closely resemble the medullary fibromas so often seen in normal kidneys of adults. It is highly probable that medullary fibromas are derived from an excess of embryonic connective tissue. It is unlikely that the excess of connective tissue is the fundamental disturbance since occasionally there is no great amount present, but it is an important factor in the subsequent pathogenesis of the disorder. It tends to become more dense and fibrous as the individual grows older, it compresses tubules and glomeruli and is one of the important factors that bring on progressive renal insufficiency.

Occasionally areas of cartilage or smooth muscle are found in the interstitial tissues (Busse, Berner). The significance of the excess of connective tissue is unknown. Possibly it is a compensatory result of the hypoplasia of the tubules.

Another conspicuous feature of the congenital cystic kidney of the newborn is the marked reduction in the number of nephrons. A great many contributors have noted this. It has been estimated that the number may be reduced to 10 per cent of the normal, but no actual counts have been made. In the medulla there are usually only a few collecting tubules. In our cases, Nos. 6, 10, and 12, no normal collecting tubules were found in the sections studied, and there were only a few in the others. Normal convoluted tubules and glomeruli are commonly found only in small islands usually in the subcapsular zone. Sometimes there are only rudimentary tubules and glomeruli between the cysts and no areas of normal cortex (Case 13). Wakely found no tubules or glomeruli in 1 case. Normally the collecting tubules form by repeated dichotomous branching of the primitive outgrowths of the pelvis, and it is evident that this process is disturbed so that only a few collecting tubules are formed, or they become detached from the main branch after their formation. Some of the cysts may represent dilated outgrowths from the pelvis, especially those with a fibromuscular wall.

The cysts are lined with a single layer of epithelium which is usually cubical but sometimes columnar, or it may be flattened so that it resembles endothelium. The cytoplasm is commonly clear and the cell boundaries may be distinct but sometimes there is a granular cytoplasm. Often the lining epithelium has proliferated to form papillary ingrowths, and it is this feature that suggested the neoplastic theory of the cystic kidney.

In some kidneys numerous cysts show a glomerulus projecting into the lumen, the glomerulus being supplied with afferent and efferent arterioles and having a normal structure. Such cysts have been interpreted as dilated capsular spaces. In other kidneys none of the cysts is supplied with a glomerulus. Beckmann, 1856, described glomerular cysts. Frequently one sees a normal glomerulus connected with a normal tubule which opens into a cyst. Apparently some of the cysts represent dilated segments of a tubule. There is abundant evidence that some cysts communicate with capsular spaces and tubules but apparently they seldom drain into the pelvis.

Von Mutach, 1895, studied a number of small cysts, 0.5 to 2 mm. in diameter, in serial sections and was able to show that each was a dilated segment of a tubule.

Hyaline glomeruli are almost invariably present in adult polycystic kidneys, but they are evidently rare in infants. There were none in our cases. Staemmler, however, noted a number of hyaline glomeruli in a stillborn infant.

The arteries and arterioles show a normal structure in all of our cases except one (Case 13), in which a definite medial fibrosis is present. This caused the media to appear somewhat thicker than normal and more homogeneous. There is no change in the intima and no narrowing of the lumen in any instance. Staemmler mentioned a thickening of the walls of the arteries in a stillborn infant which also had many hyaline glomeruli. I have found no other reference to arterial disease in the polycystic kidneys of infants.

Renal insufficiency in the newborn group is clearly due to hypoplasia of the parenchyma. There is a great reduction in the number of nephrons and the majority of those present apparently do not communicate with the pelvis. It is evident that those who survive into adult life must have originally had more normal parenchyma than is present in this group. Renal insufficiency is readily demonstrable by functional tests (Tow).

We shall now direct attention to the group of patients with cystic kidneys in which death was due to some extrarenal cause and no symptoms referable to the kidneys were present (Table VI).

Cystic Kidneys in the Adult

CASE 17. On gross examination the external surfaces of the kidneys were finely granular and the cortices were somewhat thinner than normal. There were numerous small cysts from 1 to 6 mm. in diameter scattered over the surfaces and throughout the cortical portions. There was an abundance of normal parenchyma.

Microscopically the cysts are lined by cubical epithelium and show no fibrosis or atrophy about them. There is no evidence that they are compressing the kidney by expansion. There is no indication that the disease would have progressed toward renal insufficiency.

CASE 18. The patient was troubled for a number of years with

weakness, edema of the ankles and dyspnea on exertion. There was a trace of albumin in the urine. These symptoms were attributed to the very large cystic liver found at postmortem. On section numerous cysts were found scattered throughout both cortical and medullary portions. There was abundant normal parenchyma.

Microscopically the cysts are lined by cubical or flattened epithelium. Some of the larger ones are surrounded by a zone of fibrous

TABLE VI

Polycystic Kidneys with No Symptoms Referable to the Kidneys (Subclinical Group)

Case No.	Autopsy No.	Age	Sex	Blood pressure	Cystic liver	Weight of heart	Weight of kidneys	Intimal thickening		Interstitial tissue	Cause of death
								Art-eries	Arte-rioles		
17	23-152	yrs. 85	F	120/?	—	gm. 270	gm. 110	2	0	—	Lobar pneumonia
18	23-756	61	F	124/73	+++ 2430 gm.	300	90 350 400	2	0	—	Lobar pneumonia
19	25-481	52	M	?	+	400	Twice normal	2	2	—	Cerebral hemorrhage
20	26-9	35	F	?	+	375	325 525	1	0	—	Subarachnoid hemorrhage
21	26-591	65	M	?	—	320	380 500	2	0	—	Perforated ulcer
22	27-272	52	F	?	++	235	260 190	2	0	—	Lobar pneumonia
23	29-537	33	F	172/102	—	380	500 350	2	1 —	—	Subarachnoid hemorrhage

tissue. There is atrophy of the parenchyma in narrow septa between the cysts, but none elsewhere and there is very little evidence of a progressive destruction of the parenchyma. That this is a true congenital cystic kidney is indicated by the cystic liver and the presence of cysts in the medulla. There is no increase of interstitial tissue. This case may be interpreted either as an early stage of clinical cystic kidney disease or as a moderate degree of cystic disease which would not have progressed to renal insufficiency. The latter interpretation seems more probable.

CASE 19. Sudden death. No previous illness known. On section the kidneys were filled with cysts of varying size. Both cortex and

medulla were involved. There was a large amount of normal parenchyma.

Microscopically the smaller cysts are surrounded by normal parenchyma. The larger cysts are usually surrounded by a zone of fibrous tissue, and the narrow septa between them are composed of fibrous tissue. There is some displacement of collecting tubules by the medullary cysts. On the whole there is evidence of a slow increase in size of the cysts. The arteries and arterioles show the intimal thickening characteristic of hypertension.

CASE 20. No symptoms of renal disease. The larger kidney was filled with cysts of varying size which occupied both cortex and medulla. There was a large amount of normal parenchyma. Only one pole of the smaller kidney was cystic.

Microscopically there is very little evidence of atrophy of the parenchyma except in thin septa between the cysts. The arteries are practically normal.

CASE 21. No symptoms except those referable to duodenal ulcer. On section numerous cysts of different sizes were scattered throughout the cortex and medulla. There was a large amount of normal parenchyma.

Microscopically the thin septa between the cysts are composed of atrophic parenchyma, and there are wide zones of pressure atrophy around some of the larger cysts. There is, therefore, definite evidence that the larger cysts are expanding. Some of the large cysts are lined by flattened epithelium. There is no atrophy about the small cysts. The arteries show only the intimal thickening corresponding to the age.

CASE 22. Admitted in shock. No history of kidney disease. Both kidneys contained numerous cysts in both cortical and medullary portions. There was, however, a large amount of normal parenchyma. A calculus was found in an upper calyx of the left kidney.

Microscopically there is complete atrophy of the parenchyma in the narrow septa between the cysts and external to large cysts which lie near the surface. The pressure atrophy about the large cysts indicates a slow increase in size. There is no change in the tissue surrounding small cysts. The arteries show the typical senile intimal thickening and there are a few, small, wedge-shaped areas of atrophy at the surface, due to arterial disease.

CASE 23. Some albumin in the urine. Hemorrhages in the fundi. Urea nitrogen 14 mg. per 100 cc. of blood. Virilism. Adenoma of the right adrenal 2 cm. in diameter. On section there were a number of cysts from 5 mm. to 3 cm. in diameter scattered throughout the cortex and medulla. The cysts were not very numerous and there was abundant normal parenchyma between them.

Microscopically there is a little pressure atrophy about the larger cysts. The arteries and arterioles show the intimal thickening characteristic of hypertension. In arteries that curve about large cysts a definite medial fibrosis is noted. A few small atrophic areas have resulted from the arteriolosclerosis.

The patients with symptoms referable to the kidneys are listed in Table VII. Supplementary data on each case is given in the following paragraphs.

CASE 24. The patient had noticed a mass in the left side for several years, but it was not painful. She had had two pregnancies and during each there was a marked edema of the ankles. Palpable left kidney. No indigo-carmin from either kidney in 20 minutes. Symptoms and death from bacterial endocarditis. On section each kidney was filled with closely-packed cysts from 2 mm. to 5 mm. in diameter. The largest cyst was 8.5 cm. in diameter. There were large areas of normal parenchyma scattered between the cysts (Fig. 2). Both cortex and medulla were cystic.

Microscopically the islands of solid tissue show a fairly normal structure. There is more or less complete atrophy of parenchyma in the septa between the cysts and there is a zone of pressure atrophy around the larger cysts. The arteries in the walls of large cysts show medial fibrosis with some thickening of the media but no intimal change. The atrophy is clearly due to expansion of cysts and not to arterial disease. The large expanding cysts are usually lined by a flattened epithelium.

CASE 25. Frequent burning urination, pyuria for 4 months. Left kidney palpable. Small amount of albumin in the urine. Blood urea nitrogen 18.6 mg. per 100 cc. 2 weeks before death. Pus from right ureter. At postmortem extensive suppuration was found in the larger left kidney. Both kidneys were filled with cysts separated by thin septa or islands of parenchyma.

Microscopically the islands show a fairly normal cortex except for

TABLE VII

Polycystic Renal Disease (Clinical Group)

Case No.	Autopsy No.	Age	Sex	Blood pressure	Functional tests	Cystic liver	Weight of heart	Weight of kidneys	Arteries		Arterioles		Cause of death
									In-timal thickening	Medial fibrosis	In-timal thickening	Medial fibrosis	
24	27-506	yrs. 27	F	78/52	No indigo-carmin in 20 min. PSP 30%, Urea N 18.6 mg. ?	-	gm. 240	gm. 820	1	2	0	0	Bacterial endocarditis
25	30-456	66	F	180/90		-	320	500	3	1	2	0	Pylonephritis
26	30-1790	Adult	M	?		+	480	450	2	1	0	0	Postoperative shock
27	14-139	44	F	?		+	?	600	3	0	0	2	Postoperative infection
28	15-381	52	M	?	?	-	480	450	2	2	0	2	Fracture of skull
29	14-63	33	F	?	?	++ 9510 gm.	350	550	?	?	?	?	Postoperative shock
30	32-146	64	F	210/190	?	-	285	330	3	2	0	2	Uremia
31	25-297	45	F	90/50	Sp. gr. 1010	-	292	275	1	1	0	0	Uremia
32	26-599	59	M	124/84	?	++	250	663 Very large	?	?	?	?	Uremia

33	28-1109	48	M	?	Urea N 118 mg.	-	355	520	1	2	1	1	Uremia
34	29-1159	25	M	165/110	PSP o, Urea N 174 mg.	-	420	545	1	1	0	1	Uremia
35	34-623	48	M	144/84	NPN 163.7 mg.	-	550	1240	?	?	?	?	Uremia
36	16-310	42	F	?	?	-	?	960	3	1	0	3	Uremia
37	23-92	39	F	?	?	-	?	1250	1	2	0	3	Uremia
38	31-1150	58	F	235/110	?	-	420	1280	?	?	?	?	Uremia
39	31-2054	68	F	248/140	Urea N 142.9 mg.	-	325	1300	?	?	?	?	Uremia
40	33-298	40	M	190/134	Urea N 109.9 mg.	-	560	2500	3	1	0	1	Uremia
41	33-1540	40	M	High	?	-	430	1100	0	3	0	3	Uremia
42	34-841	57	F	170/110	Urea N 234 mg.	++	275	1570	3	0	0	0	Uremia
43	31-1682	72	M	165/85	Urea N 138.3 mg.	-	780	2150	3	2	to 1	1	Uremia
44	34-1701	88	M	206/118	Urea N 85.4 mg.	-	536	1450	3	2	to 3	3	Uremia
								99	3	2	3		

PSP = phenolsulphonethylphthalein (output in 2 hrs.). NPN = non-protein nitrogen. Urea N = urea nitrogen. The numerals 1, 2, 3 show the degree of the indicated process.

leukocytic infiltration. The arteries show the hypertensive form of intimal thickening.

CASE 26. Nephrectomy for hematuria. Postoperative death. On section cysts were found throughout the kidneys, leaving only small islands of parenchyma between them.

Microscopically there is atrophy of the parenchyma between and around the larger cysts. There are small areas of subcapsular atrophy related to cysts near the surface. The arteries show a moderate amount of intimal thickening.

CASE 27. The patient had noticed a mass in the right side of the abdomen about 2 years before her death. There were attacks of painful hematuria on several occasions. The right kidney was removed. Death about 6 weeks later from infection of the surgical wound. On section the entire kidney was a mass of cysts but there were some fairly large islands of solid parenchyma.

Microscopic examination shows pressure atrophy of the parenchyma between and around the larger cysts. There are also atrophic tubules with hyaline glomeruli scattered through the solid islands of parenchyma. The large and small arteries show an extreme intimal thickening (Fig. 3) and the arterioles show a marked medial fibrosis with no intimal disease. The medial fibrosis causes the walls of the arterioles to appear thick and homogeneous. The arterial disease is apparently responsible for the hyaline glomeruli and is therefore contributing to the renal insufficiency, but the cysts are the chief cause of the parenchymal atrophy.

CASE 28. The patient died shortly after sustaining a fracture of the skull. Although no clinical data were available, the extensive destruction of the kidneys justifies the classification of this case with the clinical group. On section the kidneys were filled with cysts but there were a number of areas of solid tissue, 1 to 2 cm. in diameter, in a longitudinal section. Both cortex and medulla were cystic.

Microscopic sections show extreme atrophy of the parenchyma in the narrow septa between the cysts and around the large cysts. The solid areas show many normal tubules and glomeruli. The majority of the glomeruli show some thickening of the capillary basement membranes, and in many of them there are large hyaline areas due to fusion of the thickened membranes. Some glomeruli

are completely hyalinized. Both arteries and arterioles show medial fibrosis. The size of the heart indicated that hypertension was present, and this may be responsible for the thick glomerular capillary walls. The chief cause of the destruction of the kidney is expansion of cysts, but a contributory cause is a slow hyalinization of the glomeruli.

CASE 29. Enlargement of abdomen for 3 years. Urine normal. An enormous cystic liver (9510 gm.) was found at postmortem. Small areas of parenchyma were noted between the cysts in the kidneys. No renal tissue was preserved for microscopic study.

CASE 30. The patient died in coma following a fracture of the humerus. The urine was bloody. On section all parts of the kidneys were filled with cysts 3 mm. to 2.5 cm. in diameter. There was practically no persistent parenchyma (Fig. 1).

The septa between the cysts show occasional normal glomeruli and tubules, but for the most part they are composed of dense fibrous tissue with remnants of hyaline glomeruli. The arteries usually show a marked fibrous intimal thickening and medial fibrosis. The arterioles show chiefly a marked medial fibrosis. The changes in the vessels suggest disuse atrophy. The numerous hyaline glomeruli in the septa are probably the result of pressure atrophy.

CASE 31. Duration of symptoms about 9 weeks; weakness, anorexia and loss of weight. The urine showed a trace of albumin. Several convulsions on the day before death. On section the parenchyma of the kidneys was almost completely replaced by cysts, only small islands of cortex near the capsule were found.

Microscopic sections through the solid areas of cortex show large glomeruli and large dilated tubules that are normal, except for the hypertrophy. There is marked atrophy of the parenchyma between and around the cysts. The arterioles are normal and the arteries show only the usual changes associated with age. Small areas of interstitial fibrosis are seen in which the connective tissue is too abundant and dense to be explained as a mere replacement fibrosis from atrophy of tubules. Atrophy seems to be due entirely to expansion of the cysts.

CASE 32. The patient had not been well for 10 years, but had been acutely ill for only 3 months. He complained of weakness, loss

of weight, enlargement of the abdomen with a dull aching pain in the upper part, and hematuria. He had had several attacks of hematuria during the preceding 18 months. One brother and one sister have some form of renal disease. There was a marked secondary anemia. Only small islands of cortex were found in the kidneys which were almost completely replaced by cysts. No renal tissue was preserved for microscopic examination.

CASE 33. The patient stated that he had felt well until August 4th, 3 weeks before death. He complained of weakness and dizziness and soon became irrational. He was in coma the last few days of his life. At postmortem a glioma, 7 cm. in diameter, was found in the right frontal lobe. On section the kidneys showed only a few small islands of cortex. There were thin septa between the cysts.

In the islands of intact cortex the arterioles show the hyaline intimal layer characteristic of hypertension, but the destruction of parenchyma is due entirely to the cysts and not to arterial disease. The cysts show papillary epithelial ingrowths. The glomeruli show a definite increase of endothelial cells and some thickening of the capillary basement membranes.

CASE 34. Five years before his death the patient sustained fractures of the pelvis, ribs and legs in an accident. The urine was bloody at that time. One year later he began to have attacks of pain in the right flank, accompanied by nausea, vomiting and weakness. Seven months before his death he had a severe attack and was admitted to the hospital. There were many red blood cells in the urine and the phenolsulphonephthalein output was zero. The blood urea nitrogen was 128 mg. per 100 cc. at that time. Subsequent study showed no special features. The albumin in the urine varied from a trace to a large amount. There was some blurring of vision. The eyegrounds showed some tortuosity of the arteries but no hemorrhages or exudates. The kidneys were palpable. On section the kidneys showed only a few islands of persistent cortex. The cysts were separated by thin septa and many of them contained blood.

Microscopically no normal parenchyma is found. The cysts are usually surrounded and separated by dense fibrous tissue which contains only remnants of tubules and hyaline glomeruli or is purely fibrous in structure. In the larger areas between the cysts there is an

interstitial fibrosis and the majority of the glomeruli are either entirely hyaline or they show patches of hyaline degeneration due to thickening of the capillary basement membranes. The changes in the glomeruli are not due to obstruction of the arterioles; they are probably caused by pressure of the cysts or by focal glomerulitis.

CASE 35. The patient had occasional attacks of pain in the right flank for 2 years preceding his death. On March 5, 1934, he had an attack of hematuria which lasted several days. Both kidneys were enlarged and the right was tender. Death, April 6, 1934. On section the parenchyma of the kidneys was almost completely replaced by cysts, and many of the cysts were filled with purulent or gangrenous material. No tissue from the kidneys was preserved.

CASE 36. The patient died 3 hours after admission to the hospital and had not been under medical care previously. She was in coma and had seven convulsions during 3 hours. The urine contained a large amount of albumin. On gross examination of the kidneys a few small, irregular islands of cortex were found. There was pus in some of the cysts.

In the solid areas there is a marked interstitial fibrosis. Many of the tubules are atrophic but others are large and dilated. There are many hyaline glomeruli surrounded by old fibrous crescents. The arterioles show advanced medial fibrosis, but their lumens are large (Fig. 4). The destruction of the parenchyma is not due to arterial disease, but to the cysts, interstitial fibrosis and an old glomerulonephritis.

CASE 37. The patient complained of weakness, abdominal pain, headache and dizziness. She had a convulsion shortly before death. On section of the kidneys only small, irregular areas of parenchyma were found.

These areas show severe interstitial fibrosis in some parts. The few normal tubules are greatly hypertrophied. There is no evidence that atrophy is due to arterial disease. There is a marked medial fibrosis of the arterioles (Fig. 5). The hyaline glomeruli seem to be due to pressure atrophy of the tissue resulting from expansion of the numerous cysts.

CASE 38. The patient had had hypertension for about 10 years. She had been in as good health as usual when she became uncon-

scious during the night and died the following day. She was not paralyzed. The urine showed a specific gravity of 1008 and a trace of albumin. The parenchyma of the kidneys was almost completely replaced by cysts varying in diameter from 1 mm. to 4 cm. No renal tissue was preserved for microscopic study.

CASE 39. The patient had complained of visual disturbances for many years. During the past 2 years she had had dyspnea, swelling of the ankles, dizziness and hypertension. Many attacks of nausea and vomiting during the past year. Polyuria during the fall of 1931. Admitted to the hospital Dec. 17, 1931. Died 2 days later. Hemoglobin, 55 per cent. Moderate albuminuria. The kidneys were filled with cysts and contained very little solid parenchyma. No renal tissue was preserved.

CASE 40. The patient had an attack of hematuria about 3 years before his death. He had no other symptoms until Nov. 20, 1932, when he developed weakness and edema of the ankles following a "cold." Later he developed dyspnea and nausea which persisted. Hemoglobin 65 per cent. Slight albuminuria. Death, Feb. 13, 1933. On section the kidneys consisted almost entirely of cysts separated by thin septa. The largest solid areas of parenchyma were less than 1 cm. in thickness.

There is a very marked interstitial fibrosis. No areas of normal tissue are found microscopically. The arteries show a pronounced intimal thickening. The arterioles show hyaline degeneration of the media but no narrowing of their lumens. There is no evidence that any atrophy is caused by vascular disease; the growth of the cysts and the fibrosis of the interstitial tissue seem responsible for the destruction of the parenchyma.

CASE 41. The patient developed a sudden paralysis of the right arm and leg in August, 1930. He was under hospital care for hemiplegia and hypertension. The kidneys were palpable. There was a large amount of albumin in the urine toward the end of his illness. Death, Sept. 15, 1933. On section the kidneys showed only occasional small masses of solid tissue between the cysts.

Microscopic sections of these areas show extreme fibrosis of the interstitial tissue. The arteries and arterioles show a very marked fibrosis of the media, but no narrowing of the lumens. There are

some normal glomeruli. The hyaline glomeruli are apparently the result of pressure from the dense interstitial tissue.

CASE 42. In March, 1934, the patient first consulted a physician because of loss of weight, dyspnea and swelling of the ankles. At this time the hemoglobin was 60 per cent, there was a very low excretion of phenolsulphonephthalein, and the blood pressure was 170/110. She was admitted to the hospital on April 17, 1934. The urine showed albumin +, many pus cells and a specific gravity of 1010. May 6th, stupor and muscular twitching developed. Death, May 8, 1934. On section the parenchyma of the kidneys was almost completely replaced by cysts. No islands of normal parenchyma were found.

Microscopic sections of small solid areas between the cysts show a marked interstitial fibrosis and extensive atrophy of parenchyma, but there are some large normal glomeruli and tubules. The arteries show an extreme elastic-intimal thickening which narrows the lumens to a marked degree. The terminal arterioles show only occasional bands of subintimal hyalin. Some of the hyaline glomeruli are apparently the result of the arterial disease, others are caused by pressure atrophy.

CASE 43. A diagnosis of "Bright's disease" had been made 12 years previously, but no details of the symptoms at that time were available except that he was confined to bed for 1 year. He was treated for gastric ulcer in February, 1930, and at that time he had a definite renal insufficiency — phenolsulphonephthalein output 20 per cent in 2 hours, blood urea nitrogen 75.6 mg. He was fairly comfortable from that time until about May 1, 1930, when he developed great weakness and vomiting. He had dyspnea on exertion and slight edema of the lower extremities. He had never noted hematuria. A severe degree of renal insufficiency finally developed. He remained in the hospital until his death, Oct. 10, 1931. In the left kidney one could not distinguish cortex from medulla. Three calyces were traced into cysts. There was no normal renal parenchyma. The right kidney, which was the smaller, showed some islands of fairly normal parenchyma. The cysts occupied both the cortex and the medulla. These were typical polycystic kidneys but they were only slightly enlarged. The heart was enormously enlarged.

Microscopically there is a severe disease of the arteries and arte-

rioles with advanced atrophy of the parenchyma due to the arterial disease. The principal change in the arteries is an intimal thickening which is largely composed of collagenous tissue. The glomeruli are in various stages of hyaline degeneration due to thickening of the basement membranes of the capillaries. Many of the glomeruli show also an endothelial increase characteristic of primary hypertension with renal insufficiency (malignant hypertension). The extremely tortuous arteries are evidence that the kidneys were originally much larger. The cysts evidently played an unimportant rôle in the destruction of the parenchyma. The best interpretation of this kidney is a primary hypertension superimposed on a cystic kidney.

CASE 44. The duration of the patient's illness was indefinite. He did not complain of dyspnea or cough but there was a moderate pitting edema of the lower extremities. The hemoglobin was 50 per cent. Albumin in the urine 0 to +. The eyegrounds showed tortuous arteries and some exudate but no hemorrhages. The heart was greatly enlarged. Coma developed and the patient died 1 week after admission. On section the thin cortex of the kidneys was closely studded with small cysts. There were no cysts in the medullary portions.

There is an extreme elastic and collagenous intimal thickening of the arteries and arterioles and the great majority of the glomeruli are hyaline or in a stage of hyaline degeneration due to thickening of the capillary basement membranes. A few glomeruli show the endothelial proliferation characteristic of primary hypertension with uremia (malignant hypertension). The cysts are for the most part dilated hypertrophic tubules (Fig. 6). Tubules may be traced into cysts, and all transitions may be found between normal tubules and large cysts. The interpretation of this kidney is primary hypertension with atrophy of most of the parenchyma and subsequent hypertrophy and dilatation of persistent tubules to form cysts. There is no evidence that it is a true congenital cystic kidney. The absence of cysts in the medulla is very significant since the medulla is nearly always cystic in the congenital cystic kidney. Greene's case of renal rickets, mentioned above, is apparently a dilatation of persistent tubules in an atrophic or hypoplastic kidney.

CASE 4. The unilateral polycystic kidney (No. 4, Table IV) may be discussed with this group. The patient was admitted to the hos-

pital, Dec. 6, 1933, for a traumatic fracture of the left tibia and fibula. Her blood pressure was 140/72 at that time. The fracture failed to heal. In April, 1934, the blood pressure was 195/110 and there was slight edema of the ankles. The blood pressure varied from time to time between 140/80 and 195/110. The hemoglobin was 85 per cent, and there was occasionally a trace of albumin in the urine. She died May 24, 1934, with signs of apoplexy. At postmortem there was found softening of the left temporal lobe from arterial thrombosis. The right adrenal was replaced by a hypernephroma weighing 435 gm. The right kidney was of normal structure. The left kidney weighed 666 gm. and was filled with cysts. There was a fair amount of normal parenchyma at one pole.

Microscopically there is atrophy around and between the cysts, but the solid areas have a normal structure. The arteries in both the normal and the cystic kidneys show a fairly marked, elastic-intimal thickening and the arterioles in both kidneys show a hyaline intimal thickening. The changes in the arteries and arterioles are typical of hypertension and there are no differences in the vessels of the two kidneys.

Changes in the Arteries and Arterioles

Several writers have commented on the condition of the arterial system in the polycystic kidney. Meader, 1907, in a stillborn infant found the arteries quite large and their walls extensively thickened. McKinlay, 1920, in a male 30 years of age, with a very high blood pressure, noted that the arterioles frequently showed great thickening and hyalinization of their walls. Staemmler, 1921, in a stillborn infant, observed thickening of the walls of arteries and arterioles but no changes in the intimal layer.

Ritter and Baehr, 1929, made an excellent study of the arterial system by injecting the vessels with a mixture of barium sulphate and gelatin and then clearing the specimen. They injected kidneys from three cases and found that the interlobular and intralobular arteries lie largely in the walls of cysts and that they are greatly elongated because of the increased size of the kidney and their tortuous course around the cysts. The injected kidneys showed a disappearance of the finer arterial branches. These investigators believed that they were dealing with a typical, well developed arteriolo-

sclerosis and that arterial disease was responsible for the destruction of the persistent islands of parenchyma in the polycystic kidney.

Podgurski, 1930, paid special attention to the arteries in 7 cases of polycystic kidneys in adults. He noted that the arteries and arterioles were "thick-walled and sclerotic." He called attention to hypertrophy of the media in 1 case, and elastic-intimal thickening in another. In 3 cases there was pronounced arteriolosclerosis and 2 of these were interpreted as true hypertension.

Braasch and Schacht, 1932, stated that there is usually a marked thickening of the arteries and arterioles. Their illustration shows an artery with no intimal disease but apparently with hypertrophy of the media.

A thorough study of our material has been made to determine the structural changes responsible for the thick walls of the vessels and the relation of this process to other forms of arterial disease. In the group of newborn infants we found normal arteries and arterioles in all instances except one. In this case the larger arteries showed a definite medial fibrosis which caused the walls to appear somewhat hyaline. There was no intimal disease. In the subclinical group, Table VI, the arterial changes correspond with the age of the patient and no effect of the cystic disease can be seen.

In the clinical group there are a number of cases in which the arterial changes are in excess of processes attributable to age. The degree of intimal thickening and medial fibrosis in the arteries and arterioles is indicated in Table VII — Grade 3 representing the maximum degree of the process.

The Arteries: (A) The intima shows a high degree of intimal thickening in 8 cases (Grade 3 in Table VII). The lesser degrees of involvement are explainable on the basis of age. The thick intima is responsible for the "thick-walled" appearance of the arteries noted by several observers. Elastic tissue stains show that the thick intima contains several coarse elastic lamellae, and the azocarmine stain shows a large amount of collagenous tissue in addition to the elastic layers. Occasionally a large artery is almost completely closed by a layer of collagenous tissue inside the elastic layer (Fig. 3). The elastic laminae may be torn and fragmented, indicating retrogressive changes. The significance of this intimal thickening is not entirely clear. It is known that hypertension tends to cause elastic intimal thickening, but the large amount of collagenous tissue fre-

quently seen suggests that some other factor is also concerned. It is possible that disuse atrophy is partly responsible for the thickened intima. In the atrophic kidneys of chronic glomerulonephritis the arteries often show a similar structure. It is believed that the intimal thickening is compensatory in that it reduces the lumen to a size consistent with the decreased blood flow. In the polycystic kidneys a large proportion of the glomeruli and tubules have disappeared, leaving only cysts and fibrous tissue which require much less blood.

(B) The media of the arteries shows some degree of fibrosis in most instances but not in excess of that found in normal kidneys of persons of corresponding age, except in one instance, Case 41, in which it is very pronounced. With a high degree of medial fibrosis the media of the artery has a glassy hyaline appearance.

The Arterioles: In the arterioles medial fibrosis is often prominent (Figs. 4 and 5). In a few instances definite hyaline masses are seen in the media. Aside from the 2 cases of true hypertension (Cases 43 and 44) there is relatively little intimal disease in the arterioles, and their lumens are widely patent. The typical arteriosclerosis associated with hypertension is characterized by a subintimal hyaline deposit. The arteriolar disease in polycystic kidneys differs from this since it is chiefly fibrosis and hyalinization of the media with a normal intima.

Case 44 is not a true polycystic kidney but a cystic dilation of persistent hypertrophic tubules in a hypertensive kidney. Case 43 is apparently true primary hypertension superimposed on a polycystic kidney. Aside from these 2 cases the arterial disease is responsible for very little atrophy in the persistent islands of parenchyma of the polycystic kidney. In fact, the arterial disease is more readily explained as an effect than as a cause of atrophy. The great increase in the length of the arteries and their tortuous courses around the cysts probably alter their structure. The other influences affecting the arteries are the increased blood pressure and the decrease of functioning parenchyma which they supply.

That the veins of the kidneys are sometimes obstructed is indicated by Duskes' report in which an extensive collateral venous circulation was described.

Hypertension in polycystic renal disease is to be attributed to increased resistance to the flow of blood through the kidneys or to anemia. This same principle applies in glomerulonephritis. The

experimental work of Goldblatt and his collaborators indicates that anemia of the kidney is more important than resistance to blood flow in causing hypertension. It is possible that obstruction in the renal circulation or anemia of the kidneys brings about reflex stimulation of the vasomotor center which results in a generalized increase of vascular tonus.

ETIOLOGY AND PATHOGENESIS

It will not be necessary to review the older theories of the origin of polycystic kidneys; we may begin with the theory proposed by Ribbert. A discussion of the earlier theories may be found in Braunwarth's paper. After the embryologists had established the separate origin of the collecting and convoluted tubules, Ribbert offered the hypothesis that convoluted tubules fail to unite with the collecting tubules and subsequently develop into cysts. This interpretation gained widespread acceptance for many years, but has now been disproved clearly by Kampmeier's investigations.

That the polycystic kidney is due to a developmental defect is indicated by the associated anomalies that are commonly present in the newborn group. One may find anencephaly, spina bifida, encephalocele, myelocele, craniorachischisis, undescended testes, club foot, anomalies of the internal genital organs in the female, and so on. Cysts are frequently found in the liver and occasionally in the pancreas also (Rümler). There is no doubt that true polycystic kidneys are always congenital in origin and definitely hereditary.

In order to understand the modern theory of the origin of the congenital cystic kidney it is necessary to be familiar with the normal organogenesis of the kidney. The primitive outgrowths from the renal pelvis divide and subdivide repeatedly to form collecting tubules. Each division of the collecting tubules is referred to as a generation, *e.g.* first generation, second generation, and so on. Each newly formed collecting tubule from the first generation onward comes into contact with the metanephric blastema — an undifferentiated cellular mesenchymal tissue. The blastema forms a cap over the end of the collecting tubule and differentiates into a curved solid structure, the primitive convoluted tubule. A lumen soon forms in the solid tubule and opens into that of the collecting tubule. Commonly the opposite end of the short convoluted tubule develops a

glomerulus. The first three or four generations of convoluted tubules are not permanent, they become detached from the collecting tubules and persist for a time as cystic structures usually provided with a glomerulus. The generations of tubules after the third or fourth are permanent. In the early embryonal period there are, therefore, numerous cysts in the kidneys formed from the first few generations of tubules. McKenna and Kampmeier have made numerous reconstructions of these cysts. Normally these fetal cysts atrophy but their persistence offers the simplest explanation of the origin of single and multiple cysts in the adult kidney.

A fundamental objection to the "failure of union" theory is that the metanephric blastema does not develop into a tubule until after it has joined the collecting tubule. At least there is no satisfactory evidence that any differentiation of the metanephric blastema occurs when the collecting tubules do not penetrate it.

The various stages in the development of the polycystic kidney have not been worked out but it is probable that the first stage is a persistence of the fetal cystic structures arising from the first generations of collecting tubules. It is possible that many of the primitive tubules fail to become detached and thus prevent the subsequent formation of collecting tubules. The rarity of collecting tubules in polycystic kidneys supports this hypothesis. On the basis of Kampmeier's theory we can understand why a cyst often contains a glomerulus in its wall and why a normal tubule frequently opens into a cyst; the detached primitive tubule differentiates into glomerulus and tubule and a part or all of the tubule becomes cystic. Since the glomeruli of the detached tubules have a normal blood supply the secretion of urine would tend to cause cystic dilatation of the tubules.

An outstanding feature of the polycystic kidney is the marked reduction in the number of functioning units or nephrons. When death occurs in early infancy it is found that the number of normal nephrons is insufficient to maintain life. But since a great many persons with polycystic kidneys live until middle life or later we may ask — what was the structure of their kidneys at birth? And what changes did they undergo between birth and the final termination in uremia? We have only incomplete answers to these questions — the various stages in the development of the polycystic kidney have not been identified. We know that the kidneys become progres-

sively larger. Numerous writers have observed great increases in size over a period of years or months. It is also fairly clear that this increase in size is due largely if not entirely to an increase in the size of the cysts and not to an increase in number. The stages immediately preceding renal insufficiency have been identified; our subclinical group (Table VI) contains examples of this type. These kidneys differ from those with renal insufficiency in that they show larger areas of parenchyma not yet replaced by cysts. The continued expansion of the cysts in this subclinical group would destroy more parenchyma and bring on renal insufficiency. Stieda described an excess of connective tissue in the medulla in a subclinical case. Although the stages have not been identified it is highly probable that polycystic kidneys at birth have a still greater proportion of intact parenchyma than is found in the subclinical group at postmortem. In childhood and youth the persistent islands of parenchyma hypertrophy and compensate for the tissue destroyed by the cysts. This is probably the reason why there are so few deaths between infancy and the third decade. But in adult life the ability to hypertrophy is greatly decreased, parenchyma is destroyed more rapidly than it can be replaced and the patient progresses toward renal insufficiency.

The destruction of parenchyma is due largely to the cysts. An associated hypertension, as in Case 43, or glomerulonephritis as in Case 36, may destroy the islands of parenchyma, but these complications are unusual. The destructive effects of pressure are easily observed between and around the cysts. The tortuous thick-walled arteries and the elevated blood pressure probably contribute to some extent to the destruction of the parenchyma. The expansion of the cysts produces a pressure atrophy of the tubules and glomeruli. The abundant interstitial tissue, especially in the medulla, becomes more dense and fibrous with age and also compresses the tubules. Some of the smaller cysts in adult kidneys represent hypertrophy of persistent tubules, or they may be tubules that originally communicated with the pelvis which were later obstructed by the pressure of connective tissue or cysts. The great majority of the larger cysts are probably of fetal origin.

Cystic kidneys are frequently found in the horse and pig, rarely in the cat and wild animals (Hartoch).

SUMMARY

Polycystic kidneys are found once in about every 500 post-mortems, and from 5 to 10 per cent are unilateral.

In our autopsy service about one-third of the cases occurred in infants, the majority of which were stillborn.

There are relatively few clinical cases between infancy and the age of 25 years, but the disease is always congenital.

We may distinguish a surgical type in which the patient presents symptoms and signs referable to one kidney, *viz.* pain, tumor, hematuria, infection, and so on.

In the medical type the symptoms are those of acute or chronic renal insufficiency, and the functional disturbances correspond to those of contracted kidneys. Attacks of hematuria are, however, distinctive.

Edema is rarely prominent, and cardiac failure is unusual.

The systolic blood pressure is 150 mm. Hg or higher in over 50 per cent of the cases that have been reported, and hypertension is somewhat more frequent in advanced than in early stages of the disease.

Cardiac hypertrophy often develops but is much less pronounced than in primary hypertension.

Retinal changes of the hypertensive type may be found, especially in those with very high blood pressure.

Some patients live many years after symptoms have developed.

When the renal reserve is low, *i.e.* in advanced cases, pregnancy causes a typical nephritic toxemia, but there is no disturbance when the renal reserve is good.

There is abundant evidence that polycystic renal disease has a strong hereditary tendency.

The pyelogram is of great diagnostic value in cases where the diagnosis is otherwise difficult.

In the newborn group the outstanding structural changes are the presence of numerous cysts, hypoplasia of parenchyma, *i.e.* a great reduction in the number of nephrons, and an excessive amount of interstitial connective tissue.

The numerous "glomerular" cysts are interpreted as vestigial structures derived from the first three or four generations of tubules.

In the subclinical group there is abundant renal parenchyma

between the cysts, while in the clinical group the parenchyma may be reduced to a few small scattered islands.

The progressive atrophy of the parenchyma is brought about chiefly by continuous expansion of the cysts. Arterial disease plays a minor rôle in this process, except in the occasional case in which true primary hypertension is superimposed on the cystic disease.

The arteries usually show a marked intimal thickening which is attributed chiefly to disuse atrophy but partly to hypertension. Medial fibrosis in the arteries is explainable on the basis of age.

The arterioles show no marked intimal disease except when primary hypertension is a complication. However, they often show a marked medial fibrosis. This process is not true arteriolosclerosis.

Kampmeier's theory of the origin of the cysts is favored.

One case is described (Case 44) in which compensatory dilatation of persistent tubules in a hypertensive contracted kidney caused it to resemble the true congenital cystic kidney.

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DESCRIPTION OF PLATES

PLATE 56

- FIG. 1. Case 30 (Table VII). Section of a kidney from a patient who died of uremia. No islands of normal parenchyma are present.
- FIG. 2. Case 24 (Table VII). Section of a kidney from a patient who died of bacterial endocarditis. There was some renal insufficiency. Note the large areas of persistent parenchyma.



I

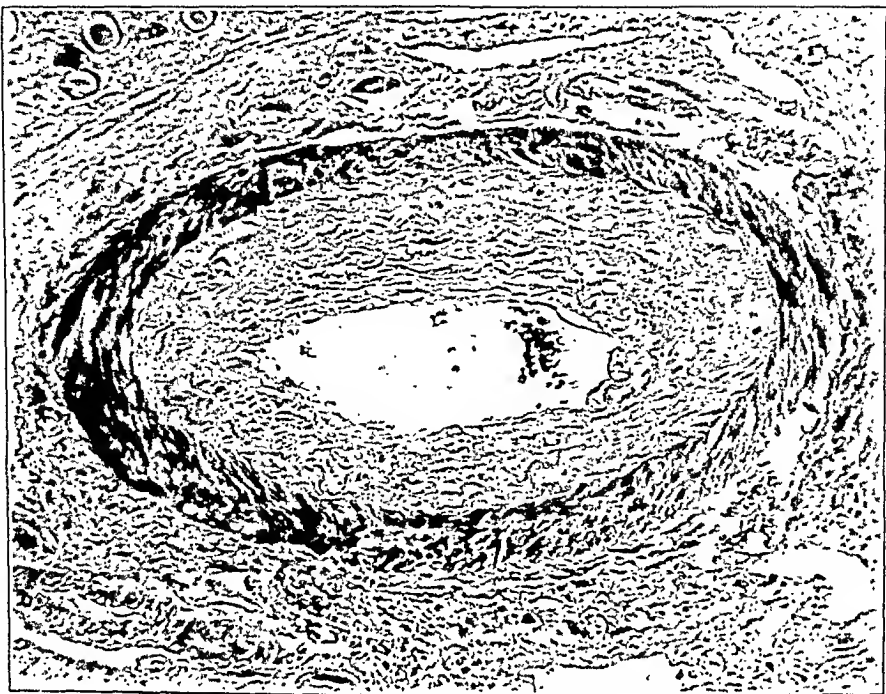


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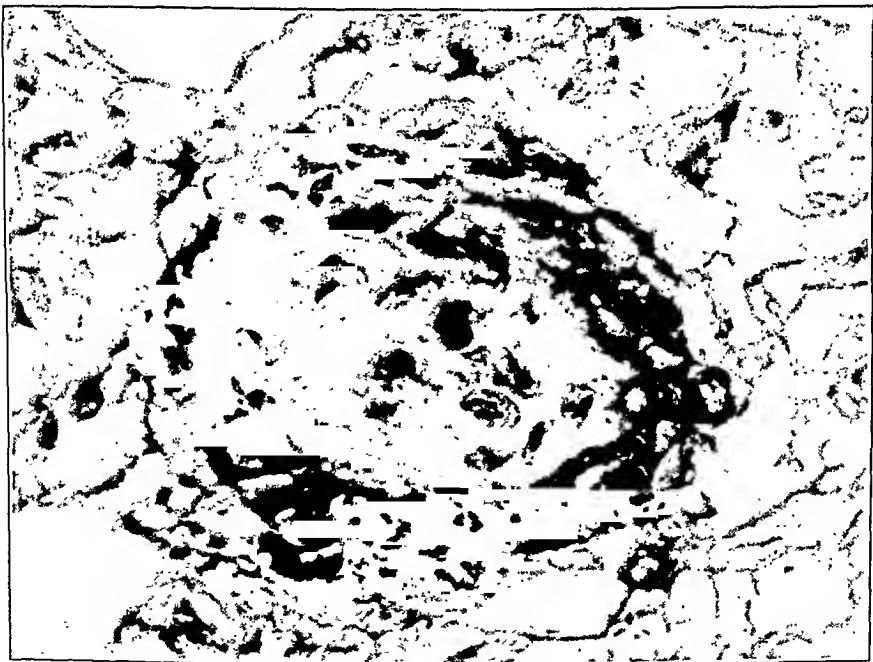
PLATE 57

FIG. 3. Case 2 (Table VII). Medium sized renal artery showing a high degree of intimal thickening. The intima is composed of both elastic and collagenous fibers.

FIG. 4. Case 36 (Table VII). Arteriole showing extreme medial fibrosis. The wall consists entirely of collagenous fibers which appear black in the illustration. There are no muscle fibers or elastic fibers. The intima is normal. Mallory-Heidenhain stain.



3



4

PLATE 58

FIG. 5. Case 37 (Table VII). Arteriole showing extreme medial fibrosis. Nuclei of muscle fibers are seen (the clear rounded areas), but no muscle sarcoplasm is present. The wall is composed entirely of collagenous fibers (black). The intima is normal. The lumen is dilated. Mallory-Heidenhain stain.

FIG. 6. Case 44 (Table VII). Hypertensive kidney with multiple cysts formed by dilation of tubules. These are not congenital cysts.



5



6

THE LIPID CONTENT OF LIVERS OF NON-IMMUNIZED AND IMMUNIZED HORSES *

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Prolonged or intensive immunization of horses for the production of therapeutic serums is so often complicated by fatty degeneration or even rupture of the liver, that a chemical and histological study was made of the tissue changes and of the extent of the degenerative processes in this organ. It is the purpose of this paper to record the observations made in the course of these studies. Further investigation of the lipid constitution of the blood, plasma and serum will be recorded in a second paper.

A number of investigations have been made of the chemical nature of the fatty changes in the liver. In 1914 Imrie¹ examined human livers. The method of procedure for the estimation of fats consisted in saponifying the tissue with potassium hydroxide. To the saponified digest, sulfuric acid was added to liberate the fatty acids, which were extracted with petroleum ether and evaporated; the residue was weighed. No corrections were made for cholesterol. It is evident that the fatty acids contained in the phospholipids are a part of the fatty acids determined. The significance of the phospholipids was not considered in Imrie's work.

Theis^{2,3,4} concluded from his investigation that there is a definite and quite constant correlation between the phospholipids and the total fatty material in normal liver tissue, and proposed the equilibrium equation $\text{phospholipid} \rightleftharpoons \text{neutral fat}$. In abnormal conditions he found that "there may be either a change in total lipid content or more generally a shift to the right of the phospholipid-neutral fat ratio."⁴ The distribution ratio in beef liver he reported as 55 per cent phospholipid : 45 per cent neutral fat. Apparently a variation of 10 per cent in the ratio was not considered significant by Theis. The lipids of two human livers accepted as normal had ratios of 50 per

* Presented at the meeting of the American Association of Immunologists, Washington, D. C., May 9 and 10, 1933.

Received for publication September 26, 1934.

cent phospholipid: 50 per cent neutral fat and of 59:41, while two abnormal fatty livers had a ratio of 45:55 and 17:83. These obtained only about 75 per cent phospholipids in the extracted solids. This may have been due in part to incomplete precipitation of phospholipids on adding acetone to the ether solution of the lipids. MacLean and Williams,⁵ also MacLean and MacLean,⁶ found that the phospholipids may form as much as 84 per cent of the total fat and questioned the presence of any true fat.

The determination of lipids in horse liver tissue, as planned for this investigation, involved the estimation of total fatty acids, phospholipids, free and esterified cholesterol, and the iodine value of the total fatty acids. The extraction of the lipids was made with boiling alcohol and ether⁷ and is recognized as the Bloor procedure. The determination of total fatty acids and phospholipids was also based on Bloor's methods.⁸ In the estimation of phospholipids, the phosphorus was determined in the petroleum-ether extract and calculated to lecithin. The phosphorus was precipitated and weighed as ammonium phosphomolybdate. The method used is essentially that described by Elek.⁹ This procedure supplanted the acetone and magnesium chloride precipitation as practiced by Bloor.¹⁰ The Osato and Heki¹¹ methods, with slight modifications, were used for the estimation of free and esterified cholesterol. The iodine values of the total fatty acids were determined by the Rosenmund and Kuhnenn pyridine-dibromide method, as practiced by Yasuda.¹² All results obtained in the lipid work were determined by micromethods. The estimation of neutral fats (triglycerides) was made by calculating the fatty acids in the phospholipids (taken as two-thirds of the weight) and the cholesterol ester (esterified cholesterol $\times 0.734$). The sum of the fatty acids in the phospholipids and the cholesterol was subtracted from the total fatty acids. The residual fatty acids multiplied by the factor 1.045 gave the neutral fat.

VARIAION IN DUPLICATE ANALYSES

	No.	Average %
Total fatty acids	49	2.9
Phospholipids	25	1.7
Total cholesterol	23	1.6
Free cholesterol	24	1.8

With a few exceptions at the beginning of the study, the analyses were done in duplicate and, in order to record the accuracy of the data, the above tabulation has been compiled.

The livers from 8 non-immunized and 41 immunized horses were studied. The immunized animals included 11 tetanus, 6 diphtheria, 1 botulinus, 7 meningococcus, 12 pneumococcus, and 4 streptococcus horses. One horse which was not immunized but had parasitic infestation died. The analysis of this liver is appended to record the changes found in this condition; likewise that of a donkey which had been immunized, first with sheep cells and then with *B. abortus*. Some of the horses had been immunized with various cultures and toxins; that used last determined the classification. Two of the horses studied were not under active immunization at the time of death and are classified as "resting." The results of the chemical analyses are recorded in Tables I and II.

TABLE I

Variation in the Lipid Content of the Livers of Non-Immunized and Immunized Horses

	Number of horses		
	6 non-immunized	2 resting	41 immunized
	<i>range</i>	<i>range</i>	<i>range</i>
Total fatty acids %	2.48-2.82	2.63-2.73	1.82-9.12
Total fatty acids iodine value. . %	90.2-96.7	97.0-104.9	77.1-103.3
Phospholipids %	3.24-4.55	3.68-3.87	1.41-4.30
Free cholesterol %	0.159-0.216	0.177-0.199	0.169-0.262
Esterified cholesterol %	0.010-0.030	0.007-0.032	0.007-0.493
Neutral fat %	0.25-0.54	0.14-0.15	0.15-5.75

The percentage of the total fatty acids in the non-immunized and resting horses varied but little; in the immunized horses the variation was quite pronounced. The iodine values indicated a quite uniform degree of unsaturation. The amount of phospholipids of the non-immunized and resting horses varied only slightly, while that of the immunized horses showed appreciable differences in percentage and, with few exceptions, was uniformly less. The free-cholesterol values in the non-immunized and resting horses were similar; with the immunized horses the range was higher. The esterified cholesterol was

TABLE II

The Distribution of Lipids in the Livers of 8 Non-Immunized and 41 Immunized Horses

Horse No.	Sex	Age	Death *	Period of immunization yrs. mos.	Total fatty acids		Phospholipids per cent	Free cholesterol		Esterified cholesterol per cent	Neutral fats per cent	Ratio phospholipids: neutral fats per cent	Ratio phospholipids: free-cholesterol	Scharlach R staining †	Titer of serum	
					Per cent	Iodine value		per cent	per cent						units	Final
Non-immunized																
417	M	28	BS		2.48	91.3	4.55	0.216	0.020	0.31	91.90	21.2:1	#			
435	F	23	BS				3.24	0.159	0.030			20.3:1				
416	M	23	S				4.05	0.202	0.017			19.8:1				
425	F	22	BS		2.82	96.7	3.87	0.196	0.014	0.25	94.60	19.8:1	+			
436	M	23	BS		2.69	95.1	3.24	0.172	0.021	0.54	86:14	19.0:1	#			
408	F	27	BC		2.58	90.2	3.50	0.191	0.010	0.25	93:70	18.2:1	#			
294	M	8	BS		2.63	104.9	3.68	0.177	0.032	0.14	96:40	20.7:1	#			
363	M	18	BS		2.73	97.0	3.87	0.199	0.007	0.15	96:40	19.4:1				
Average																
					2.66	95.9	3.75	0.189	0.019	0.27	93:70	19.8:1				
Immunized																
418	M	5	BS	10	3.35	94.8	4.30	0.235	0.021	0.49	90:10	18.2:1		660	270	
401	M	7	BS	5	2.58	95.8	3.37	0.200	0.025	0.33	91:90	16.9:1	+	10 +	10 +	
374	M	20	BS	10	2.77	98.5	3.27	0.195	0.048	0.58	85:15	16.9:1	2 +	310	180	
329	M	19	BS	2	2.67	100.0	3.39	0.210	0.033	0.40	90:10	16.2:1	#	650	460	
385	F	16	BS	10	3.08	90.3	3.31	0.231	0.020	0.91	79:21	14.4:1		1130	1130	
345	M	23	D	6	3.95	77.1	2.99	0.232	0.017	2.04	59:41	12.9:1	3 +	350	300	
320	M	20	BS	2	2.48	90.3	2.42	0.191	0.168	0.77	76:24	12.7:1	2 +	900.	410	
356	M	24	BS	1	2.22	103.3	2.52	0.204	0.122	0.51	84:16	12.3:1	+	500	300	
339	M	5	BC	1	2.76	88.4	2.46	0.262	0.276	0.96	72:28	9.4:1	+	560	300	
325	M	20	DR	2	1.83	93.5	1.63	0.214	0.138	0.67	70:30	7.6:1	2 +	850	850	
354	M	17	DR	1	1.82	96.9	1.66	0.246	0.021	0.73	69:31	6.8:1		1000	940	
409	M	17	BS	2 1/2	3.92		3.84	0.234	0.029	1.40	73:27	16.5:1		5	1 #	
412	M	17	BS	3	2.78	97.1	3.19	0.228	0.036	0.67	83:17	15.7:1		325 #	100 #	
463	F	9	D	1 1/2	7.14		2.41	0.169	0.048	5.75	30:70	14.4:1				
421	F	4	D	1 1/2	3.80		2.92	0.205	0.018	1.92	60:40	14.2:1				
155	M	21	BS	1 1/2	2.93		3.22	0.259	0.023	0.81	80:20	12.5:1		750	750	
380	M	2	D	1 1/2	2.63		2.36	0.194	0.024	1.09	69:31	12.0:1				
B. Botulinus																
271	F	20	BS	4 7	2.59	93.6	3.57	0.236	0.012	0.20	95:50	15.1:1		550	100	

Immunized																
Meningococcus	389	M	13	D	II	1.97	95.5	2.61	0.181	0.024	0.22	92.80	14.4:1	2 +	ap. stand. high	low
	287	M	18	BS	3 II	2.50	86.0	2.82	0.217	0.049	0.62	82:18	12.9:1	+	low	ap. stand. low
	352	M	3	BS	3	2.96	90.1	2.75	0.225	0.024	1.04	73:27	12.2:1	+	high	low
	370	M	15	BC	I	2.26	93.3	2.61	0.221	0.007	0.54	83:17	11.8:1	+	high	high
	375	M	17	D	10	2.63	84.5	2.43	0.205	0.031	1.03	70:30	11.8:1	3 +	high	low
	347	M	22	BDR	I II	2.79	92.2	2.33	0.255	0.229	1.12	68:32	9.1:1	5 +	high	low
	357	F	24	DR	I 10	3.27	94.5	1.41	0.261	0.294	2.21	61:39	5.4:1	4 +	high	high
Pneumococcus	448	M	15	*	8	2.62		3.22	0.210	0.025	0.47	87:13	15.4:1	4 +	ap. stand. high	ap. stand.
	359	M	18	S	2	4.71	82.1	3.92	0.253	0.030	2.17	64:36	15.4:1		ap. stand. low	low
	397	M	24	BC	I	3.10	87.6	3.48	0.231	0.018	0.80	81:19	15.1:1		low	low
	430	F	17	D	4	4.52	84.8	3.21	0.213	0.029	2.46	57:43	15.1:1		low	low
	453	M	18	D	I	2.48	93.9	2.75	0.189	0.027	0.66	81:19	14.6:1		ap. stand. low	ap. stand.
	434	F	17	BS	8	2.96	92.9	3.76	0.258	0.009	0.47	89:11	14.6:1		high	low
	342	M	5	BS	2	3.18	101.0	3.58	0.248	0.008	0.82	81:19	14.4:1		ap. stand. low	low
	451	F	21	S	6	9.12		3.33	0.238	0.098	0.71	32:68	13.9:1		ap. stand. low	low
	388	M	21	BC	8	2.35	84.6	3.29	0.238	0.015	0.15	96:40	13.7:1	+	ap. stand.	ap. stand.
	376	F	19	C	10	4.25	78.9	3.34	0.254	0.053	2.12	61:39	13.1:1	3 +	ap. stand. low	ap. stand.
	450	F	18	*	4	3.02	87.9	3.22	0.257	0.026	0.89	78:22	12.5:1		high	high
314	M	21	BS	2 II	2.42	100.1	2.84	0.230	0.037	0.53	84:16	12.3:1	=			
Strepto- coccus	364	F	17	BC	2	3.78	84.6	4.13	0.226	0.018	1.06	80:20	18.2:1	2 +	+200	+200
	283	F	19	BS	4	2.65	83.4	3.29	0.261	0.018	0.46	88:12	12.7:1	+	+200	+100
	163	F	21	DR	7 10	7.01	82.4	2.04	0.241	0.493	5.53	27:73	8.4:1		+1200	+1200
	332	F	21	D	3	2.33	94.0	2.13	0.261	0.008	0.95	69:31	8.2:1		=100	-40

Miscellaneous

Sheep am-boceptor; <i>B. abortus</i>	M	18	C	8	4.06	78.2	2.36	0.179	0.005	2.60	48.52	13.1:1	6 +		
Parasitic infestation	M	2	D		2.64	114.9	1.98	0.239	0.010	1.37	59.41	8.3:1	4 +		

* B = bled out; S = shot; C = chloroformed; D = died; DR = ruptured liver; No. 448, chloral hydrate, and No. 450, magnesium sulfate intravenously.

† The amount of fat in the liver, as indicated by staining sections with scharlach R, is arbitrarily designated as: = within normal limits; + slight increase; 2 + moderate; 3 + marked; 4 +, 5 +, 6 + very marked.

‡ Previous immunization: No. 294, 2 years 2 months, tetanus, pneumococcus, meningococcus; had been resting 10 months when sacrificed.

§ Immunization discontinued after 4 and 16 days respectively. Condition complicated by infectious anemia.

** Died of acute toxemia.

a low value with most of the horses; the few exceptions account for the wide range recorded. The neutral fats in the non-immunized and resting horses were uniform in amount and indicate a low level under conditions approximating the normal; with the immunized horses the range was much higher.

The data obtained from the analyses of these livers of non-immunized and immunized horses indicate ratios between the phospholipids and neutral fats higher than those reported by Theis or MacLean. These percentage ratios are recorded in Table II. Owing to its lower phospholipid and higher free-cholesterol content, the phospholipid:free-cholesterol ratio of liver tissue from immunized horses, with the exception of those that died during a rest period, was lower than that of liver tissue from non-immunized horses. The phospholipid:free-cholesterol ratio seems to have a definite relation to the extent of the injury resulting from immunization with bacterial toxins.

The titers of the serums are included in the table to complete the record. The antitoxic titers are expressed in standard units. The potency of the antimeningococcus and antipneumococcus serums is expressed in relation to the New York State minimum control serum as Approximately Standard, Low, or High. The antistreptococcus serum is classified with the antimeningococcus and antipneumococcus serums because it is produced by immunization with living cultures, but the titer is expressed in standard antitoxic units. The procedure of immunization, with the exception of the diphtheria horses which were experimental, follows closely that prescribed in the Standard Methods of the Division of Laboratories and Research of the New York State Department of Health.¹³

At autopsy the tissues of the organs were prepared for microscopic examination to determine the character and extent of the degenerative changes. These varied greatly but were most marked in the liver, kidney and adrenal glands. Apart from slight changes attributed to age, previous treatment, or artifacts of fixation, the livers of the non-immunized and resting horses were considered to approximate normal; the parenchyma to be quite so. The chemical analyses gave a uniform ratio approximating the average of 19.8:1 between the phospholipids and the free cholesterol. In so far as practical, therefore, these horses afforded a basis for comparison with the changes that were observed in the other groups of immunized horses, which had varying degrees of parenchymatous degenerative change.

In some instances this appeared to be more marked in the kidney than in the liver, but in others, and especially in horses with ruptured livers, the reverse was true. Areas of hemorrhagic extravasation were especially marked in these livers.

The most advanced and extensive lesions in the liver were found in the horses that died with rupture of the liver. Less than 10 per cent of the cells retained any of their structure in horse No. 163; in No. 325 the nuclei, cytoplasmic reticulum and granular elements had disappeared and were replaced with hyaline material stained faintly. In others the cytoplasm had degenerated in a similar manner, but the nuclei had survived. The parenchymatous degeneration affected the liver cells quite generally. Fatty infiltration, varying in degree throughout the liver, appeared in zones of the lobule or in areas. There were foci of degeneration and necrosis infiltrated with round cells, resembling the focal necrosis observed in typhoid fever. Also, changes suggesting early stages of cirrhosis were noted occasionally. The degenerative changes in the liver cells affected the periphery first and the basal portion and the nucleus last. The outer third or two-thirds of the cell may separate, forming threads which, when stained with hematoxylin and eosin, resemble the amyloid liver but do not take the characteristic stains until the advanced stages are reached. The amyloid thus appears in this hyaline material. The extremes of early toxic degeneration following a single fatal dose of diphtheria toxin and of late amyloid change are recorded respectively in the photomicrographs of horses Nos. 380 (Figs. 3 and 4) and 163 (Figs. 8 and 9). In some instances the hyaline material is derived from the blood following stasis and degeneration.

Owing to the fact that these horses had been under immunization for varying periods of time and with varying quantities of toxic material, it is only possible in this series to note the fact that the most pronounced and most advanced changes in the liver were found in those horses with the lowest ratio of phospholipids : free cholesterol.

The ratio of phospholipids : neutral fat varies quite markedly in the different groups of immunized horses. The ratio of phospholipids : free cholesterol was practically uniform in the non-immunized and resting horses. In these two groups this ratio corresponded closely, whereas among the animals under active immunization the phospholipids were reduced, and the phospholipid : free-cholesterol ratio, in general, corresponded with the changes in the liver; the reduction

in this ratio was most marked in the 5 horses that had ruptured livers.

Finally, a striking variation was noted in the esterified cholesterol. This was greatly increased in horse No. 163, which had been immunized for more than 7 years with streptococcus and died of a ruptured liver. The blood serum had consistently a very high antitoxic titer (1200 units per cc.).

Frozen sections of some of the livers which had been hardened in formalin were stained with scharlach R. The results are recorded in Table II to indicate, in general, the amount of fat observed with this stain. The disposition of fat in the liver cells of these immunized horses was interesting in that there appeared to be stages of the degenerative process in which fat globules within the cell accumulated, increasing in size. In later stages they were not so apparent, yet the fatty substance had increased, as shown by chemical analyses and by the scharlach R stain.

Correlation of the changes in the liver tissue of the immunized horse with the results of the chemical analysis is complicated by the protective action of the immunization which was, in varying degree, adequate or inadequate. This, however, is undoubtedly what occurs in the course of prolonged human infections in which the liver is involved. In a few instances an early stage of cirrhosis appeared to be associated with, or to follow, degeneration, necrobiosis and necrosis of the parenchyma. Although the cells throughout the liver suffered quite generally, the character and extent of the parenchymatous degeneration varied greatly in the different horses. The liver cells were protected as a result of the prolonged immunization. Despite extensive injury and even necrosis, many cells survived, and the nucleus appeared to be less vulnerable than the cytoplasm.

SUMMARY

Analytical data are recorded indicating the distribution of the lipids in the livers of 6 non-immunized, 2 resting, and 41 immunized horses: 11 with tetanus toxin; 6, diphtheria toxin; 1, botulinus toxin; 7, meningococcus cultures; 12, pneumococcus cultures; and 4 with combinations of streptococcus cultures and toxin.

The degenerative changes that developed in the liver as a result of the immunization are described and are recorded in the photomicrographs.

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DESCRIPTION OF PLATES

PLATE 59

FIG. 1. Horse No. 435. Female, 23 years, weight 950 pounds. Not immunized; bled (7000 cc.) twice; received one dose of 2000 units of tetanus antitoxin. January 1933, bled out (15,500 cc.) and shot. Liver weighed 5920 gm.

Section stained with hematoxylin and phloxine shows approximately normal liver. $\times 150$.

FIG. 2. Horse No. 385. Female, 16 years, weight 1150 pounds. Immunized with tetanus toxin 10 months, doses of 0.7 to 350 cc., the maximum, given at intervals of 4 to 8 days the last 3 months. Oct. 28, 1932, prolapse of rectum; sacrificed by bleeding out.

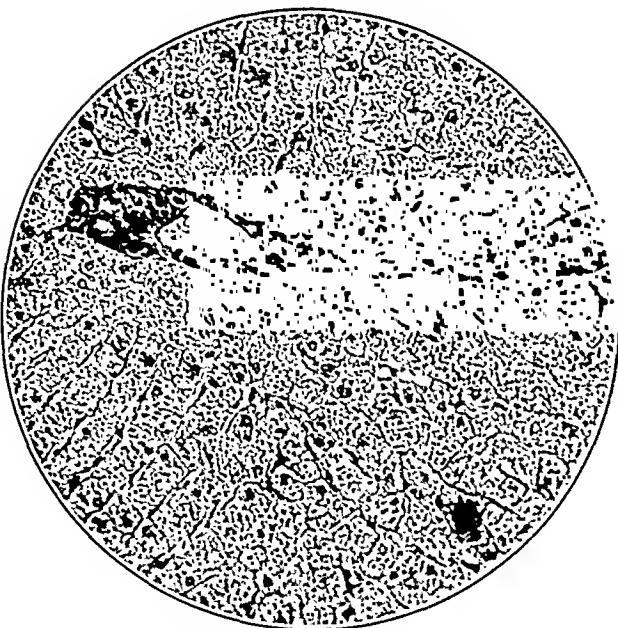
Autopsy: General condition good, medium fat; thrombosis of branch mesenteric artery; large and small intestine thickened, edematous with hemorrhagic areas; subendocardial hemorrhages in left ventricular valve; liver weighed 6400 gm.

Section stained with hematoxylin and phloxine shows degenerative change of moderate degree. $\times 150$.

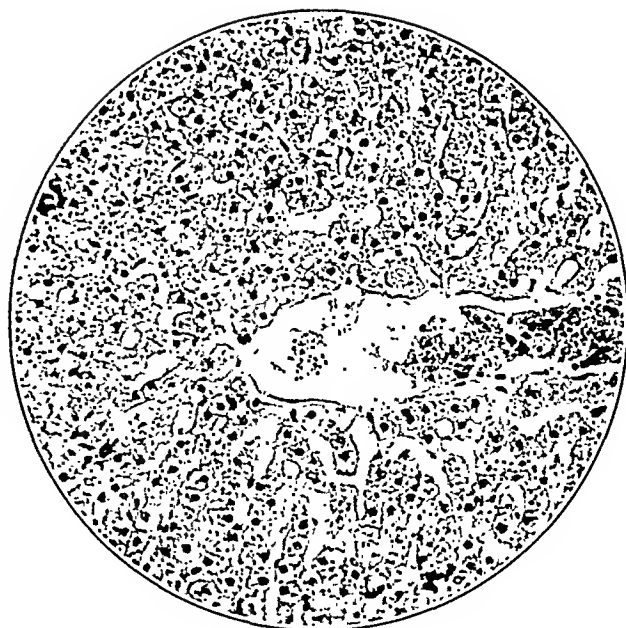
FIG. 3. Horse No. 380. Male, 2 years, weight 525 pounds. Dec. 14, 1933, received tetanus antitoxin and 6 cc. of diphtheria toxin diluted 1:20 (150 MLD) subcutaneously, followed by swelling of both sides and along abdomen. Dec. 28, 1933, found dead.

Autopsy: Petechiae in small intestine, pleura, and pericardium; congestion and edema of lungs; kidney and spleen somewhat congested; liver weighed 7075 gm.

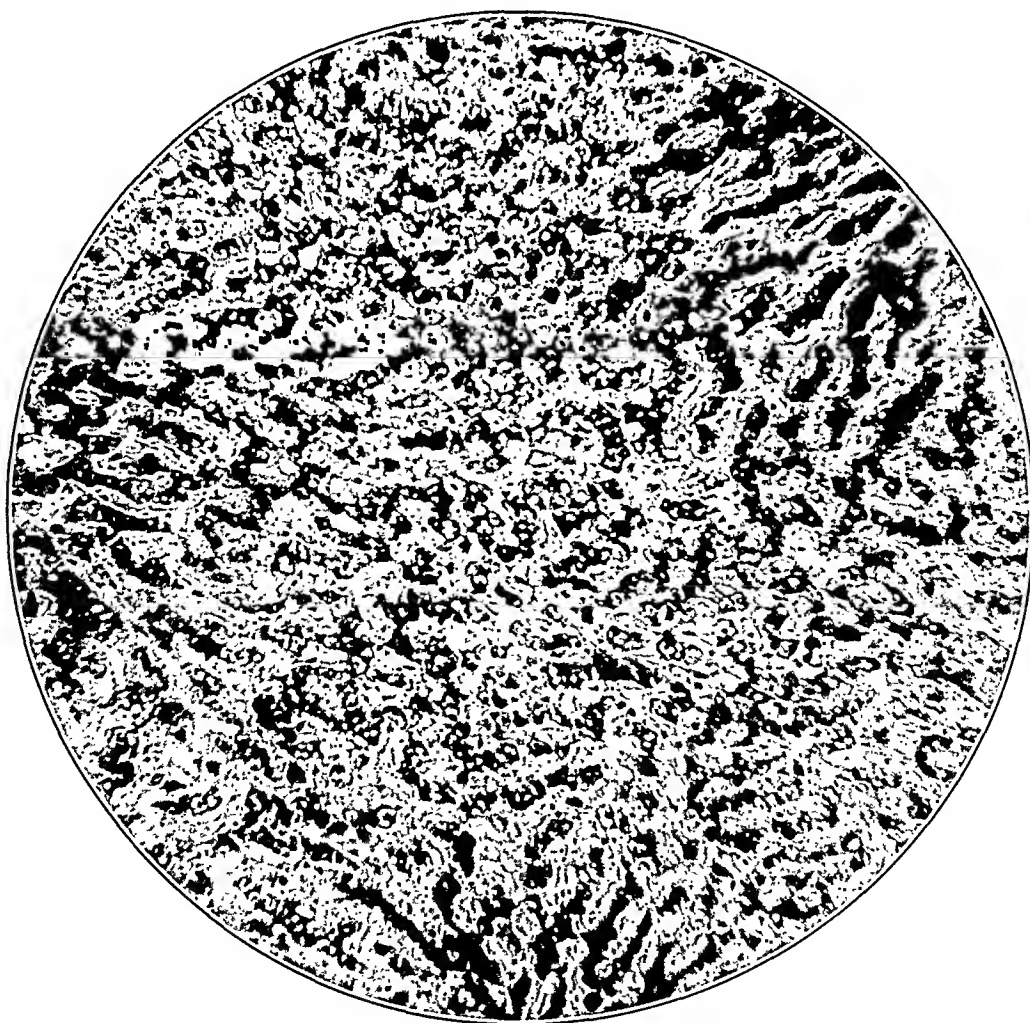
Section stained with hematoxylin and phloxine shows the marked changes of an acute fatal toxemia in a young horse. $\times 150$.



1



2



3

PLATE 60

FIG. 4. Higher magnification of Fig. 3. $\times 500$.

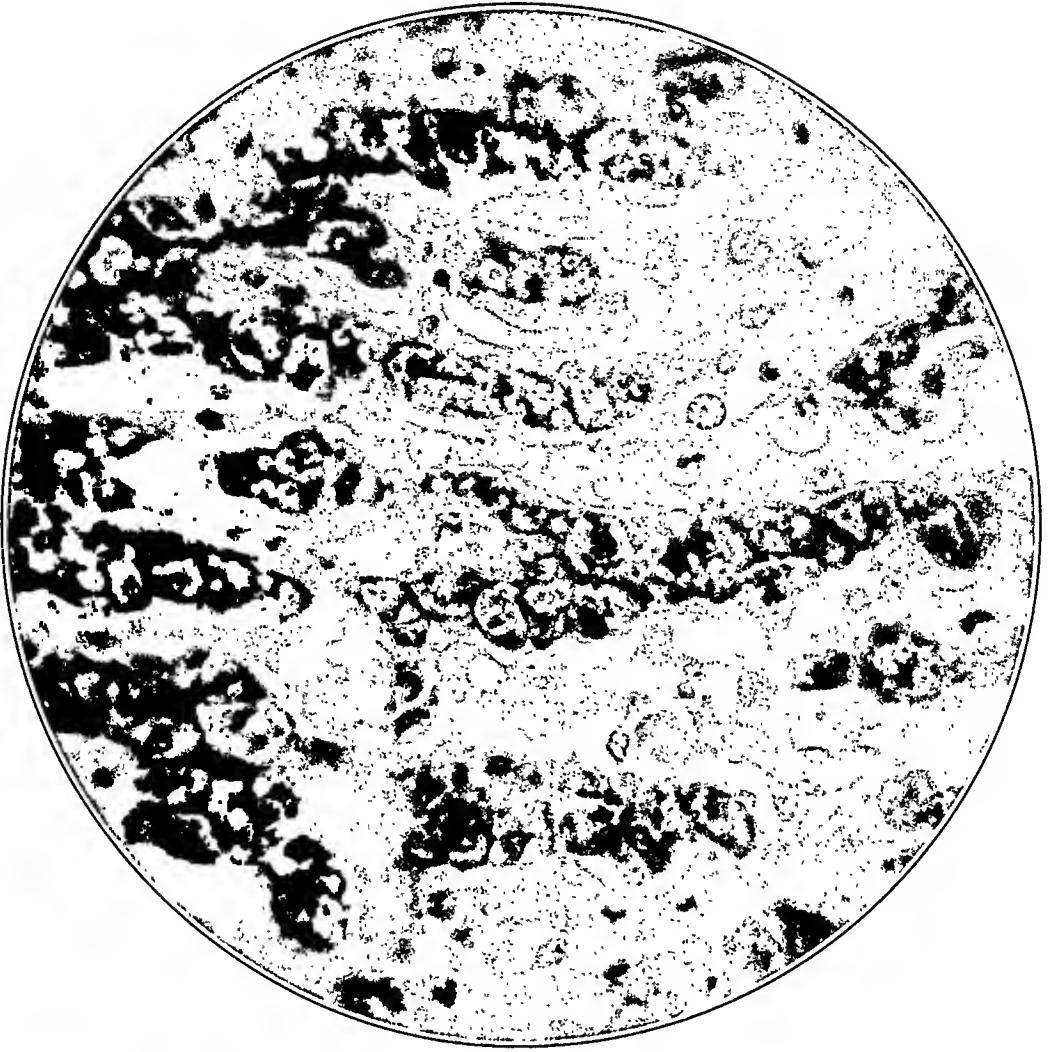
FIG. 5. Horse No. 451. Female, 21 years, weight 925 pounds. Immunized with pneumococcus type I (Neufeld strain) living cells for 6 months, with doses of 25 to 225 cc., a maximum, followed by four doses of whole culture containing 2 per cent blood, 100 cc. each. A month later, Nov. 6, 1933, shot.

Autopsy: Vegetative endocarditis, mitral and aortic valves; marked petechial eruption noted in various tissues and organs; liver, brownish yellow in appearance, weighed 7160 gm.

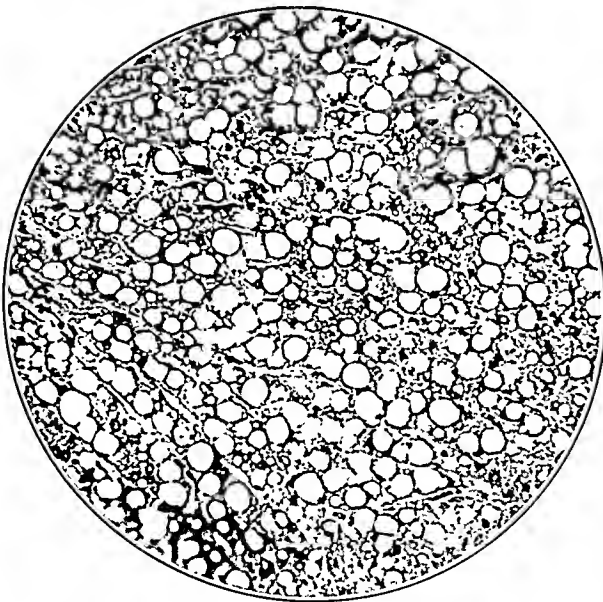
Section stained with hematoxylin and phloxine shows changes of more marked degree than those in Fig. 3. $\times 150$.

FIG. 6. Horse No. 339. Male, 5 years, weight 777 pounds. Immunized with tetanus toxin 1 year and 4 months, doses of 0.7 to 200 cc., a maximum, given the last 12 months at intervals of 4 to 8 days. July 25, 1932, sacrificed by bleeding out under chloroform anesthesia owing to poor condition and low titer of the serum.

Section stained by hematoxylin and phloxine shows advanced degenerative processes with appearance of early stage of amyloid change which, however, does not stain in a characteristic manner with iodine and sulfuric acid, methyl violet or iodine green. $\times 150$.



4



5



6

PLATE 61

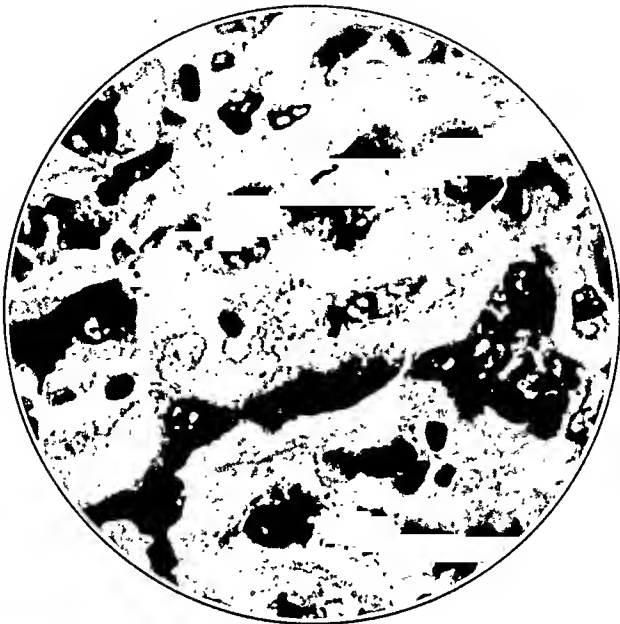
FIG. 7. Higher magnification of Fig. 6. $\times 500$.

FIG. 8. Horse No. 163. Female, 21 years, weight 900 pounds. Immunized, beginning June 1924, with streptococcus (Dochez, N. Y. 5) living culture in agar for 12 months, then supplemented by toxin in doses of 10 to 525 cc., a maximum. Rested July 1926 to August 1927. Immunization resumed through June 1928; horse then rested until April 1930, when immunization was again commenced. May 6, 1932, 7 A.M., found dead.

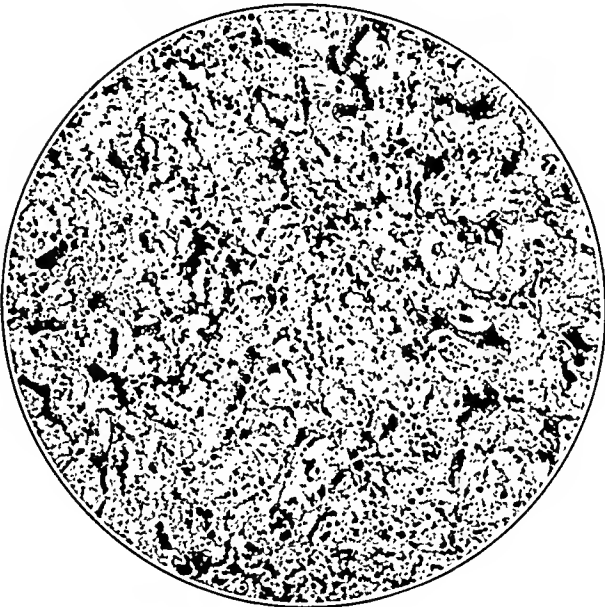
Autopsy: 11:30 A.M. Hemorrhage into abdominal cavity from large ruptures of the liver, margins and dorsal surface of right and middle lobes; liver tissue soft and light colored; marked degeneration throughout.

Section stained with hematoxylin and phloxine shows the most advanced degenerative processes observed. The hyaline material was practically all amyloid and stained a typical reddish violet with methyl violet. $\times 150$.

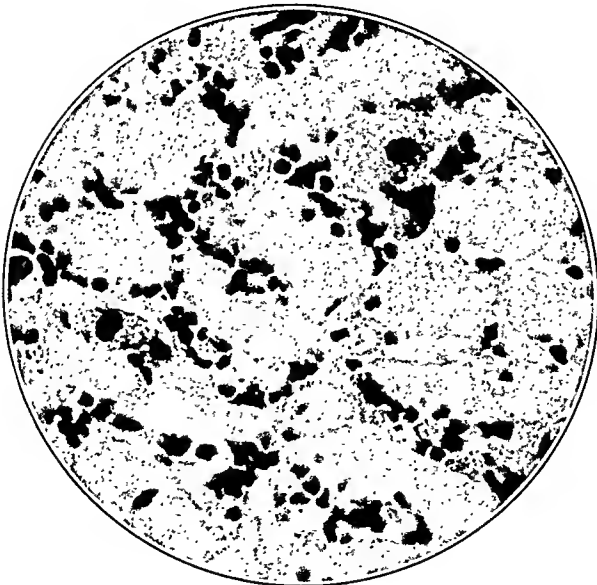
FIG. 9. Higher magnification of Fig. 8. $\times 500$.



7



8



9

A GANGLIONEUROMA IN THE NECK OF A CHILD *

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Neoplasms of nervous origin are always interesting because of their comparative rarity, their doubtful origin, their variable structure, the difficulty of properly naming and classifying them, and the uncertainty of their clinical behavior. The particular tumor whose description follows was therefore welcomed and the result of its study made the starting point of a thorough literary and critical survey of the group of tumors to which it was finally assigned.

REPORT OF CASE

Clinical History: A. C., a white female, 7 years of age, whose parents are living and well, was delivered by a normal labor, weighing at birth $9\frac{1}{2}$ pounds. She was breast fed for 1 month only.

The first teeth erupted at 3 months but the teeth of the first dentition were all poor in quality. Infancy and early childhood were uneventful, but at $5\frac{1}{2}$ years of age, a swelling made its appearance in the region of the cervical lymph nodes and continued to enlarge until, her parents becoming apprehensive, she was brought to the hospital. She was thought to have tuberculous adenitis and was given a course of five X-ray treatments, 3 weeks after the last of which she was admitted to the Hahnemann Hospital on Feb, 16th, 1931.

On admission the patient was rather pale, though apparently well nourished. Examination showed the breathing to be slightly obstructed and the tonsils enlarged. A large, smooth, nodular mass was present in the right side of the neck which was not tender, and did not fluctuate. Blood examination on admission showed hemoglobin 70 per cent, erythrocytes 3,500,000, leukocytes 5900. A second blood examination made March 4th showed the hemoglobin to be 72 per cent, erythrocytes 4,290,000 and leukocytes 6200, of which 58 per cent were polymorphonuclears, 35 per cent lymphocytes, and 7 per cent transitionals. The temperature varied between 97 and 99.8° F., and the pulse between 76 and 118.

On Feb. 23rd, 1931, a tonsillectomy was performed and a pharyngeal abscess opened. The general condition then improved, though no change occurred in the swelling in the neck and the temperature continued to rise occasionally.

An examination of the chest made March 5, 1931, showed bronchial breathing, more tubular than usual, in the midportion posteriorly, with broncophony

* Received for publication November 2, 1934

and well transmitted tactile fremitus and râles. Heart normal. A Mantoux test was performed on the right forearm and reported positive.

An X-ray examination of the chest made March 10, 1931, showed distinct thickening of the roots of the lungs with extension to both bases and to the right upper lobe. There were a few calcified areas at the roots of the lungs and some mottling of both upper lobes. The disturbance at the roots of the lungs was thought to be in the bronchial lymph nodes.

On March 12, 1931, another Mantoux and a von Pirquet test were performed on the left forearm, and on March 18th both were reported negative.

On April 4th the patient was released on the mother's request, and the diagnosis recorded as tuberculous adenitis.

She was readmitted to the hospital on April 23, 1932. Blood examination gave 7400 leukocytes, of which 61 per cent were polymorphonuclears, 38 per cent lymphocytes and 1 per cent basophiles. Two days later the hemoglobin was 91 per cent, leukocytes 6200, of which 82 per cent were polymorphonuclears, 13 per cent lymphocytes, 2 per cent transitionals and 3 per cent eosinophils. After two more days there were 65.5 per cent polymorphonuclears (55.5 per cent mature, 7 per cent immature and 3 per cent metaleukocytes), 28 per cent lymphocytes, 4.5 per cent transitionals, and 2 per cent eosinophils, with abundant platelets, many broken, poorly stained and degenerated white cells. The red cells were normal. The temperature, 100° F. on admission, descended to 99° F. that night, and remained at that point.

On April 27, 1932, an X-ray examination of the chest showed the conditions previously reported, except for pulsation in the right infraclavicular region thought to be characteristic of tuberculous infection.

On May 4, 1932, the patient was operated upon and a firm encapsulated tumor about the size of a lemon was removed from a position deep down in the neck. At the upper pole it appeared to be attached to some bony structure; elsewhere it was free.

Specimen from the operation consisted of two masses of tissue, one 6 by 5 by 4 cm., the other 5 by 3 by 2.5 cm., weighing 90 gm. They were white, fairly firm and easy to section, but the gross appearance was not characteristic of any recognized condition. There were no areas of caseation and no suggestion of tuberculosis.

After the operation the temperature of the patient varied about 100° F. for a week or so, then gradually descended to reach normal by May 20th. Additional X-ray treatments were given for prophylaxis against recurrence, on May 24th and May 27th, and the wound having healed the patient was discharged June 18th, 1932.

She reported at the hospital for follow-up examination June 24th, when she looked well and said that she felt well.

She was again seen on May 5th, 1934 (2 years later), and seemed to be quite well. Her mother, however, said that she coughed during the day and was nervous during the night. She also turned her head to one side when swallowing, probably because of the effect of the operation scar.

Careful examination of the scar and its neighborhood showed no evidence of any recurrence of the tumor. She has, however, a curious soft swelling, sharply limited to the left side of the tongue, thought by the mother to have developed after leaving the hospital. An X-ray examination of the chest showed no sign of metastatic tumors in the lungs.

MICROSCOPIC EXAMINATION

The tumor consists of a fibrillar background or stroma in which are scattered cells and cells in groups.

The fibers that make up the greater part of the tumor present considerable diversity of appearance. Some are extremely fine and wavy, others coarse and collagenous. The indistinct bundles of fibers intertwine so that they are always cut both longitudinally and transversely. Some have their respective fibrils widely separated, as in edema, others are compact. There is an occasional tendency to hyalinization, and at many points a granular breaking down followed by colliquation necrosis leading to the formation of minute indefinite spaces.

The nuclei of the fibrillar tissue are elongate, oval and vesicular where the fibers are coarse; elongate, slender and more uniform where they are fine and wavy. In many places distinct palisades of nuclei show indubitably that the fibers belong to nervous tissue. A few definite nerve fasciculi are seen, but may belong to antecedent nerves about which the tumor has grown. Except in them, no medullary sheaths were found.

The cells distributed through this background of fibers seem to form an ascending series that begins with small cells not unlike lymphocytes and ends in typical ganglionic nerve cells. The various forms — neurocytes, neuroblasts, sympathoblasts and ganglion cells — occur together. Individual cells in all or any of these stages of development are scattered singly or in groups throughout the whole tumor.

Small cell groups of spindle shape, and composed of a few large unmistakable nerve cells, with abundant cytoplasm, beautiful vesicular nuclei and large distinct nucleoli, flanked by smaller cells tapering off to very small ones at the ends of the spindle, not infrequently occur in the intervals between the fibers.

Larger collections of cells constitute a striking picture. A good many correspond with the ganglionic nerve cell groups characteristic of ganglioneuroma, and adjacent to them palisade arrangement of the nuclei shows the fibrillar tissue to consist of Schwann cells. There is no doubt but that the tumor is a ganglioneuroma. But it is not without its eccentricities. Many of the cells are immature forms closely or loosely massed together in a very delicate or loose stroma,

or in indefinite spaces in the stroma. These cell aggregations, so numerous, so large, and so indefinite, misled some of those who first examined the tissue into the error of believing that they were looking at some form of malignant epithelial tumor.

The cells represent all of the stages of development, but instead of each progressing regularly to the ganglion cell stage, those of all stages seem to multiply at random, then degenerate or liquify.

Scarcely a nerve cell, primitive or advanced in development, appears to be in a state of good health. Large ganglionic cells with beautiful vesicular nuclei commonly have finely or coarsely vacuolated cytoplasm, or they possess two, three or four nuclei, uniformly developed and healthy; or, one or several nuclei may appear normal while others may be mitotic, pyknotic or vacuolated. Mitoses, not frequent, may be found in the cells of the same group. In adjacent groups there may be none. Judging by this criterion the growth of the tumor should have been slow and should have progressed by multiplication of cells, now here, now there.

The retrogression and colliquation of the ganglion cells was attended by finer, then coarser vacuolation, then fraying at the edges. In some cases there was cytophyknosis and karyopyknosis in which the nuclei became small, dark colored bodies eccentrically situated toward the surface of the cell whose cytoplasm was solid, uniform and eosinophilic.

The general impression resulting from the study of sections stained by hematoxylin and eosin, iron hematoxylin, Weil's and Bielschowsky's methods may be summed up as follows. The neoplasm is a ganglioneuroma whose development began with the multiplication of embryonal neurocytes, and continued through the continued multiplication of those primitive cells and their evolving descendants up to the stage of ganglion cells. Whether perfected ganglion cells can multiply is uncertain, but many which seem to have reached perfection contain two, three and four nuclei and show an occasional mitotic figure. These ganglion cells probably give off neuraxons, which account for the nerve fibrils brought out by the Bielschowsky stain, and seem to excite the proliferation of the Schwann cells which show the palisades of nuclei. Then the ganglion cells, and many of the sympathoblasts not yet that far developed, lose their vitality, retrogress and dissolve into the jelly-like accumulations by which the collections of dying cells are surrounded. In a few

instances the dead cells calcify so that occasional, small, irregularly rounded aggregations of lime salts occur in the tissue. The generations of cells that have matured, produced fibrils and disappeared, account for the neurofibromatous stroma or matrix of the tumor.

COMMENT

Wahl¹³⁷ in speaking about ganglioneuroma in his excellent and complete paper credits its name "ganglioneuroma" to Odier⁹⁷ in 1803, its origin from sympathetic ganglia to Günsburg⁵⁹ in 1845, and its position with respect to other nervous tumors to Virchow in 1863. With the subsequent publications of Loretz,⁸⁴ Key,⁸ and Weichselbaum¹⁴⁰ the tumor became a well established and generally recognized entity that has attracted more and more attention and led to the reporting of more and more cases with the passage of time, as shown in the papers of Wahl,¹³⁷ Hook,⁶⁶ Rapp,¹¹⁰ Pick and Bielschowsky,¹⁰⁶ Dunn,⁴² von Fischer,⁴⁶ Riggs and Good,¹¹² Smirnoff,¹²⁸ and Bigler and Hoyne.²⁰

It is interesting to see that the number of reported cases increased from 33 in 1911, 36 in 1913, and 68 in 1932, to the present total of 143 that can be drawn from our bibliography. Accuracy regarding the number of published cases is impossible because of the difference of opinion as to just what tumors shall be included under the name ganglioneuroma. Ever since the tumor was first described by Odier the criterion for its identification seems to have been the presence of an abnormal number of ganglion nerve cells, but Gibberd⁵³ has described as ganglioneuromas two tumors in which no ganglion cells were found in the sections examined by Mr. R. Davies-Colley, his pathologist.

Pick and Bielschowsky¹⁰⁶ in 1911 expressed the opinion that the tumors of the group to which the ganglioneuromas belong, originate through embryonal malformations or the displacement of multipotential embryonal neurocytes, and consist of "ripe" or "unripe" neuroblasts. This idea agreed with that of Brossok²⁵ in 1911 and Dunn⁴² in 1915, and was elaborately discussed, especially with reference to the benignancy and malignancy of the tumors by von Fischer⁴⁶ in 1922.

According to von Fischer the primitive nerve cells, or sympathogonia, as they multiply to form tumors may maintain their primi-

tive or original shape and indicate their nature solely by the formation of the finest fibrils which lie between the cells without any kind of definite arrangement. A tumor of this structure is called "sympathogonioma" by Kohler, and constitutes the most primitive variety of neuroblastoma. With a slightly more advanced stage of differentiation these fine fibrils are gathered into coils or skeins about which there is a more or less distinct arrangement of the sympathogonia to form rosettes, while elsewhere numbers of the cells are advancing in size and differentiation to sympathoblasts or the antecedents of the ganglion cells. A tumor of this slightly higher structure is called "sympathoblastoma." When the number of sympathoblasts begins to exceed the number of sympathogonia, and more definite ganglion cells appear, singly or in groups, amid bundles of fibers and cells of Schwann, the tumor becomes "ganglioneuroma simplex."

It thus appears that two entirely different appearing tumors, the sympathoblastoma (neurocytoma of Marchand ⁸⁹ and Wright ¹⁴²) and the ganglioneuroma, simply represent the beginning and terminal stages in the neoplastic development of the embryonal nerve cells of which they are made up.

But the vegetation and differentiation of the cells do not regularly parallel one another. The cells may remain in the stage of sympathogonia or neuroblasts, when the tumors, purely cellular, highly malignant and metastatic, are easily mistaken for small round cell sarcomas; or some of them may persist in that primitive state while others differentiate into ganglion cells with nerve fiber and Schwann cell additions, giving rise to tumors, parts of which seem to be of one kind, other parts of another kind. Such a tumor was described by Robertson ¹¹⁶ as a ganglioneuroblastoma.

As the respective malignancy or benignancy of the nerve cell tumors is the result of the failure of the cells to mature on the one hand, and the perfection of their maturation on the other, any tumor containing sympathoblasts (or neurocytes) may be considered as malignant, or potentially malignant, in proportion to the number and vegetative activity of the primitive cells it contains. It is the small size of the primitive cells, their independence, and the ease with which they can be transported that are responsible for the metastases. It may therefore be assumed that every metastasis consists primarily of such primitive elements. But just as at the

primary seat of occurrence many of the cells progress in differentiation and some reach the final stage of ganglion cells, so in the metastases some or many of the cells may advance to complete differentiation and some or many ganglion cells be found in them. It may even be possible for all of the cells to complete the differentiation to ganglion cells incapable of multiplication so that an originally malignant tumor may become benign. Such a case was studied by Cushing and Wolbach.³⁸

The tumors are further divided into *ganglioneuroma immaturum* and *ganglioneuroma imperfectum*. These names explain themselves.

It is interesting that the ganglion cells sometimes seem to retain the power of multiplication until complete specialization is attained. Many of the cells, whose appearance suggests maturity, may be found in mitosis, or to have two or many nuclei.

Ganglioneuromas of the central nervous system have their histological structure increased in complexity through the presence of neuroglia elements of all kinds and in all stages of development. These constitute a special group of tumors to which the name ganglioglioneuroma has been applied.

But most peripheral ganglioneuromas also contain neuroglia-like cells, Schwann cells and nerve fibers.

AGE INCIDENCE

Ganglioneuroma may occur at any age. Von Fischer⁴⁶ found one in a stillborn infant. The tumor studied by Clegg and Moore³³ was present when the child was born. It is frequently said to be a tumor of childhood, but of 98 cases with age data we find 33 to have been less than 10, and 64 more than 10 years of age. Five of the cases in our bibliography were beyond 60 years of age, *viz.* Guizetti⁵⁸ 57 years, Brüchanow²⁶ 65 years, Bianchi¹⁸ 68 years, Uyeyama¹³⁴ 69 years, Friedrich⁴⁸ 73 years, and Weichselbaum¹⁴⁰ 79 years.

SEX INCIDENCE

It is also said to occur more frequently in female than male patients, and for this there seems to be some reason, as in 99 cases with sex data 56 occurred in females and 43 in males.

ANATOMICAL DISTRIBUTION

The left side of the body was affected in 22 and the right side in 16 cases. Many of the tumors, especially the mediastinal and retroperitoneal, are without information as to the side of the body in which the tumor originated.

The anatomical distribution is so general that ganglioneuromas may be encountered almost anywhere. The cases referred to in our bibliography were distributed as follows:

I. ABDOMINAL

(1) INTESTINAL

Poate and Inglis ¹⁰⁷

(2) MESENTERIC

Bland-Sutton ²¹

Goodhart ⁵⁶

Jones ⁷¹

MacNaughton-Jones ⁸⁷

Paterson ¹⁰¹

(3) PANCREATIC

Bianchi ¹⁸

(4) PELVIC

Beneke ¹⁴

Chiari ²²

Newmann ⁹⁵

Pick ¹⁰⁵

Schorr ¹²⁵

Stoeckel ¹³⁰

(5) RENAL

Bigler and Hoyne ²⁰

(6) RETROPERITONEAL

Babcock ⁹

Berner ¹⁷

Busse ²⁹

Cappell ²¹

Chiari ²²

Cripps and Williamson ³⁷

Fabris ⁴³

Falk ⁴⁴

Fischera ⁴⁷

Glockner ⁵⁵

Heinrici ⁶⁵

Hortolomei, *et al* ⁶⁷

Jergesen ⁷⁰

Kopřiva ⁷⁷

Krecke ⁷⁹

McFarland ⁸⁵

Miller ⁹³

Oelsner ⁹⁸

Ohse ⁹⁹

Rapp ¹¹⁰

Rosenbach ¹¹⁸

Sato ¹²⁰

Schleifstein ¹²¹

Soyka ¹²¹

Strada ¹³³

Wegelin ¹³⁹

(7) SACRAL

Chiari ²²

Günsburg ⁵⁹

(8) SUPRARENAL

Bigler and Hoyne ²⁰

Brüchanow ²⁶

Buzni ³⁰

Dalton ³⁹

Dunn ⁴²

Gamna ⁵⁰

Geller ⁵¹

Hook ⁶⁶

Jaffé ⁶⁹

Oberndorfer ⁹⁵

Peters ¹⁰⁴

Ribbert ¹¹¹

Schmidt ¹²²

Wahl ¹³⁷

Wassmund ¹³⁸

Weichselbaum ¹⁴⁰

(9) EXACT SITE NOT KNOWN

Arpino ⁶

Bartlett ¹¹

Behan ¹²

Beneke (coeliac) ¹⁴

Roman and Arnold ¹¹⁷

Smirnoff ¹²⁸

II. CEPHALIC

(1) CEREBRAL AND CEREBELLAR

Achúcarro¹
 Arpino⁶
 Berblinger¹⁵
 Bielschowsky¹⁹
 Cushing and Wolbach³⁸
 DeJong⁷²
 Dumas⁴⁰
 Katzenstein⁷⁴
 Lhermitte and Duclos⁸³
 Marinesco⁹¹
 Olivecrona¹⁰⁰
 Pick and Bielschowsky¹⁰⁶
 Robertson¹¹⁶
 Schmincke¹²⁴
 Uyeyama¹³⁴

(2) CRANIAL NERVES AND GANGLIA

(a) Trigeminal

Benda¹³
 Cooper³⁵
 Fabris⁴³

(b) Gasserian

Günsburg⁵⁹
 Hackel⁶⁰
 Haenel⁶¹
 Marchand⁹⁰
 Risel-Zwickau¹¹⁴

(c) Ocular

Krauss⁷⁸
 Perls¹⁰³

III. CERVICAL

Benda¹³
 DeQuervain¹⁰⁸
 Freund⁴⁹
 Friedrich⁴⁸
 Geymüller⁵²
 Glinski⁵⁴
 Harbitz⁶³

Haven and Weil⁶⁴

Loretz⁸⁴

MacAuley⁸⁵

Martius⁹²

Riggs and Good¹¹²

Shirai¹²⁷

Sommerfelt¹²⁹

Stout¹³²

Von Fischer⁴⁶

Woods¹⁴³

IV. FACIAL

Clegg and Moore³³

Dunn⁴²

Key⁸

V. PERIPHERAL

(1) FLANK

Wilmoth, Bertrand and Patel¹⁴¹

(2) KNEE

Hagenbach⁶²

(3) SKIN

Kredel and Beneke⁸⁰
 Montgomery and O'Leary⁹⁴

VI. MEDIASTINAL

Babcock⁹

Bergonzi¹⁶

Bigler and Hoyne²⁰

Brunner²⁷

Ranzi¹⁰⁹

Rosenson¹¹⁹

Riggs and Good¹¹²

Scott and Palmer¹²⁶

Von Rindfleisch¹¹³

VII. THORACIC

Borst²²

Guizzetti⁵⁸

VIII. VASCULAR

Anschütz⁷

Jacobsthal⁶⁸

From this summary of 127 cases it will be found that seventeen of the reported tumors were, like ours, situated in the neck. The case reports, accompanied by the necessary data, show eleven of the patients to have been children and four adults. The cervical tumor of earliest occurrence was in von Fischer's⁴⁶ case of a stillborn in-

fant; that of latest occurrence, Friedrich's ⁴⁸ case in a woman aged 73 years. The average age of the affected children was 5 years, of the adults 40 years. Five of the tumors were said to have been on the left side, five on the right.

Our case, therefore, adds one more to the seventeen reported cervical ganglioneuromas, one more to the eleven tumors reported as occurring in children and one more to those occurring in the right side of the neck.

SINGLE AND MULTIPLE TUMORS

Ganglioneuromas usually occur singly, but may be multiple, and when so the tumors may be either in close relationship with one another, widely separated or generally distributed. In the case reported by Knoblauch ⁷⁶ there was one tumor in the facial-auditory region at the anterior end of the body, and another in the sacral region at the posterior end. Other multiple tumors have been reported by Haven and Weil, ⁶⁴ Henrici, ⁶⁵ Kredel and Beneke, ⁸⁰ Knauss, ⁷⁵ Montgomery and O'Leary, ⁹⁴ Roman and Arnold, ¹¹⁷ Risel-Zwickau, ¹¹⁴ and Soyka. ¹³¹ When there are many widely distributed tumors the condition is frequently spoken of as ganglioneuromatosis, and the distributed lesions may be systematic or symmetrical.

Systematic multiple ganglioneuromas to the number of eleven, all in connection with the cranial nerves were observed by Risel-Zwickau. ¹¹⁴

Symmetrical multiple cases have been reported by Günsberg, ⁵⁹ Clegg and Moore, ³³ and by Kredel and Beneke, ⁸⁰ whose patient had about 160 separate tumors, and Montgomery and O'Leary, ⁹⁴ in whose case the skin of the patient was studded with cutaneous ganglioneuromas on the trunk and extremities, while the vermiform appendix removed at operation showed increase of ganglion cells. The patient studied by Knauss ⁷⁵ had about sixty subcutaneous ganglioneuromatous nodules scattered over the trunk and thigh. Lhermitte and Duclos ⁸³ observed a case with multiple larger tumors whose occurrence, preceded by pigmentation of the skin, seemed more like von Recklinghausen's disease than ganglioneuromatosis and raises interesting questions as to the relation between neurofibromatosis and ganglioneuromatosis, a matter beyond the scope of this paper.

Ganglioneuromas also sometimes occur in association with tumors of other kinds. Thus Hackel⁶⁰ observed one associated with meningioma, and Bianchi¹⁸ one intimately associated with carcinoma of the pancreas.

GROSS APPEARANCES

The physical qualities and gross appearances of ganglioneuromas are not sufficiently characteristic to enable the diagnosis to be made without the aid of the microscope. They are of all sizes up to that of a human head. They are usually rounded, nodular, more or less definitely encapsulated, sometimes firmer, sometimes softer. Cystic ganglioneuromas have been reported by Kopřiva⁷⁷ and Poate and Inglis.¹⁰⁷

The primitive types are softer and more uniform because of the greater proportion of cells, the mature forms more fibrillar because of fiber formation by both nerve cells and Schwann cells, and the associated formation of reticulum and collagen bundles. The cut surface usually presents a distinct fasciculation. There may also be porosity which results from the degeneration of whole groups of the ganglion cells, sometimes before, sometimes after their maturation.

PROGNOSIS

The prognosis can be made only through microscopic examination, and even with its aid it is difficult to foretell what will happen. Judgment must be based upon the developmental stages attained by the majority of the cells found. The more primitive and embryonal the cells, the more malignant the tumor; the more differentiated they are, the more benign. Unfortunately, as has already been pointed out, the same tumor may show both primitive and perfected types of structure, as in Case 2 of Beneke,¹⁴ the cases of Dunn⁴² and Martius.⁹² Such cases must be looked upon with suspicion.

Malignancy, or what has been described as malignancy, is usually shown by metastasis. Cases with metastases have been reported by Beneke,¹⁴ Berner,¹⁷ Bianchi,¹⁸ Brossok,²⁵ Busse,²⁹ Chiari,³² Jacobsthal,⁶⁸ Miller,⁹³ Pick,¹⁰⁵ and Wahl.¹³⁷ The case reported by Key⁸ is included by some critics, excluded by others.

In the case reported by Beneke¹⁴ the tumor was made up chiefly of embryonal cells and the metastases, also composed of very small cells, were in the lymph nodes and vena cava. Metastases to the liver were found by Jacobsthal⁶⁸ and Wahl¹³⁷; to the kidneys by Wahl¹³⁷ and Pick,¹⁰⁵ who also observed one on the surface of the diaphragm. There seem to be no cases of metastases to the lungs.

As the tumors seem frequently to be of multicentric origin and systematic distribution, a certain amount of caution must be exerted in judging whether multiple tumors result from metastasis. For example, the multiple tumors of the skin reported by Montgomery and O'Leary⁹⁴ can no more be thought of as metastatic than those of von Recklinghausen's disease.

TREATMENT

The literature seems to make no mention of recurrent ganglioneuromas. Most of the patients whose tumors were accessible and surgically removed seem to have been cured. Failures resulted when unexpected complications arose. There is no evidence that treatment by X-ray or radium is of value.

SUMMARY AND CONCLUSIONS

The tumor described is a well characterized ganglioneuroma. In it, however, nerve cells of all stages of development from neuroblasts to ganglion cells occur, and among them is a stroma made up of Schwann cells and nerve fibers.

It occurred in the neck of a little girl, and seems to be the twelfth case of its kind to be placed on record.

Three years after operative removal the patient is living, with no return of the tumor and no metastases.

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DESCRIPTION OF PLATE

PLATE 62

- FIG. 1. Low power view of the general structure of the tumor with the neurofibromatous background and scattered, small collections of nerve cells.
- FIG. 2. A field showing a ganglion of normal appearance with adjacent nerve and Schwann fibers and cells at one side, with an overgrown, degenerating and calcifying mass of cells opposite.
- FIG. 3. Nerve cells in various stages of development up to that of ganglion cells.



3

PRIMARY CARCINOMA OF THE LUNG *

A PATHOLOGICAL STUDY

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INTRODUCTION

Primary carcinoma of the lung is exciting an ever increasing interest, both because of its alleged rising incidence and because of the improvement in surgical technique allowing the cure of this disease in its early stages. The purpose of this paper is to classify the pathological anatomy of 69 autopsied cases of lung carcinoma occurring at the Boston City Hospital from Jan. 1, 1900 to August 1, 1934. A classification based on the microscopic morphology has been utilized in an attempt to determine the malignant potentialities, incidence by age and sex, and peculiar characteristics of different types of these tumors. It is to be hoped that a more accurate diagnosis and prognosis may be made from histological study of biopsied or expectorated tissue from these carcinomas in the future.

In 1912 Adler¹ published an analysis of 374 cases which had been reported in the literature up to that time. Of recent years, and especially since 1920, the literature abounds with references and historical reviews. For an adequate discussion of the historical, anatomical, pathological and clinical aspects of this subject, the reader is referred to the works of Adler,¹ Pilcher and Brindley,² Barron,³ Vinson,⁴ Weller,⁵ Brunn,⁶ Brockbank⁷ and, more recently, Fried,⁸ Gillespie⁹ and Hill.¹⁰

CLASSIFICATION

Little or no uniformity exists in the classification of primary lung carcinoma. Thus, older authors divided their cases into tumors arising from (a) the epithelium lining the bronchi, (b) the epithelial cells forming the mucous glands, and (c) the epithelium said to line the pulmonary alveoli (the air sacs). More recent investigators deprecate a histogenetic classification and utilize a histological stand-

* Received for publication September 27, 1934.

ard. Adler, in 1912, expressed the view that most lung carcinomas were bronchiogenic in origin, and more recently Fried has thoroughly reviewed the subject and concluded that all primary lung carcinomas originate from the lining mucous membrane of the bronchi. Certainly if non-bronchiogenic carcinomas exist they are of rare occurrence. A classification based on the gross pathology seems to lend little clarification to the subject.¹¹

For the present study the histological classification used by Weller,⁵ Rogers,¹² and in part by Fried,⁸ and other recent writers, has been utilized. The pleomorphism so frequently mentioned in relation to these tumors was occasionally found but presented no serious barrier to classification. The 69 carcinomas of this series have been divided into three large groups, namely, (a) squamous cell carcinomas, (b) adenocarcinomas, and (c) undifferentiated carcinomas. These will be discussed more fully below.

Squamous Cell Carcinomas: These tumors consist of the typical large cells with large clear nuclei and prominent single nucleoli, arranged in a manner suggesting the squamous epithelium seen in patches in the lining of the bronchi. Forty-two per cent (29 cases) were of this type and constituted the largest single group. Twenty-eight per cent of these tumors showed the formation of epithelial "pearls." Metastases were usually of the "squamous" type and rarely showed cornification or "pearl" formation. In 55 per cent the primary tumor was located in the left lung and in 44 per cent in the right lung. Forty-four per cent were in the upper lobes, 38 per cent in the lower lobes and 18 per cent at the hilum. The lobes were involved with the following frequency: left upper 20 per cent, left lower 24 per cent, right upper 24 per cent, and right lower 14 per cent. Thirty-one per cent of the total number presented as a mass at the hilum and 61 per cent either involved or occluded a primary or secondary bronchus.

Grossly the primary tumors were single, indurated, gray or white solid masses in 75 per cent of cases. Cavitation occurred in 17 per cent. Multiple masses in one lung were found in 8 per cent. Areas of tumor necrosis were relatively infrequent.

Adenocarcinomas: These tumors are very difficult to classify. Among them are tumors which undoubtedly in the past have been classified as "alveolar cell" carcinomas because their histological structure resembles that of fetal lungs. They comprise 24 per cent

(17 cases) of the entire series. Composed for the most part of cuboidal or cylindrical cells arranged in acinar formation, their chief distinguishing feature appears to be the secretion of mucus and their resemblance to bronchial mucous glands. Fifty-three per cent showed definite secretory function and 47 per cent were functionless. These will be referred to henceforth as mucinous and non-mucinous adenocarcinomas.

The non-mucinous type occurred in 8 cases, 4 of which showed a papillary adenomatous arrangement, and in three of these latter tumors ciliated columnar cells were found resembling very closely the columnar cells lining the bronchial tree. The possibility of these cells being remnants of normal bronchi was considered, but the presence of ciliated cells in the metastases of two of these tumors and the similarity of the cells to adjacent, non-ciliated tumor cells tend to eliminate this possibility. The four remaining adenocarcinomas showed no distinguishing histological feature, except a slight tendency to assume a "squamous-like" structure where the alveoli were compressed. However, they were not true acanthomas. The resemblance of these tumors to fetal lungs is suggested but not striking, and in view of the cilia found in tumors not unlike these it is believed that they are less well differentiated bronchiogenic carcinomas.

The primary tumors in the non-mucinous adenocarcinomas occurred equally in the right and left lungs. Thirty-seven per cent occurred in the upper lobes, 37 per cent at the hilum, only 12 per cent were in the left lower lobe and none were in the right lower lobe. Twenty-five per cent were in the right upper lobe and 12 per cent in the left upper lobe. In 12 per cent, diffuse multiple masses involved the whole of the right lung. Primary bronchi were involved or occluded in 37 per cent of cases and were negative or not described in the postmortem descriptions of 63 per cent.

The mucinous type of adenocarcinoma has been described at length in the past and has been called variously carcinoma myxomatodes (Willert¹³), colloid carcinoma and gelatinous carcinoma. Their striking resemblance to bronchial mucous glands and the presence of a mucoïd secretion within the alveoli serves to mark them as a characteristic lung carcinoma. They are composed of cuboidal and cylindrical cells and careful search will nearly always reveal scattered cells in secretory phases. Their structure is most frequently

that of a malignant adenoma, although true adenocarcinomas are not rare. Their metastases in the majority of instances show mucinous secretion. However, non-secreting metastases occur.

Mucinous adenocarcinomas occurred in 9 cases and in 44 per cent the primary tumors were located in the left lung and in 55 per cent, in the right lung. Fifty-five per cent occurred in the upper lobes, distributed as follows: left upper lobe 33 per cent, right upper lobe 22 per cent. Eleven per cent each occurred in the left lower, right middle and right lower lobes. In 11 per cent the tumor encircled the lower end of the trachea and right bronchus. In 55 per cent a large bronchus was involved or occluded, while in 45 per cent the bronchi were negative or not described.

In gross the adenocarcinomas appeared most frequently as gray, firm, scirrhous tumors with scattered areas of softer, yellowish tissue frequently showing grossly visible mucus. Necrosis was uncommon but when present occurred in the papillomatous carcinomas. In 94 per cent the primary tumors were a single mass occupying a lobe, the hilum or both.

Undifferentiated Carcinomas: This type is composed of the "small cell" tumors which in the past have frequently been called sarcomas. Barnard,¹⁴ Maxwell,¹⁵ and more recently Karsner and Saphir,¹⁶ have shown that these tumors are in reality carcinomas. Karsner and Saphir conclude that they are carcinomas because of their cellular arrangement, absence of reticulum, vascularization, connective tissue relations, gross characteristics resembling obvious carcinomas and distribution of metastases. Barnard describes the microscopic picture as follows. "The cells are in the main oval and when cut transversely, round and have little cytoplasm. The nuclei are oval and the majority have a distinct chromatin net with chromatin nodes, but in others the whole nucleus is so deeply stained that the nuclear structure is obscured." These tumors have been called "oat cell," "oat seed cell," small cell, spindle cell and round cell tumors in the literature.

Thirty-three per cent of the carcinomas in this series were of this undifferentiated, small cell type. Fifty-six per cent occurred in the left lung and 43 per cent in the right. Only 43 per cent of these primary tumors could be localized by lobes and they were distributed as follows: left lower and left upper lobes 13 per cent each, right upper lobe 9 per cent, and right middle and lower lobes 4 per cent

each. In 9 per cent the entire left lung was infiltrated and in 4 per cent the entire right lung. In 22 per cent the primary tumor was at the hilum of the right lung and in 24 per cent at the hilum of the left lung. The primary tumor exhibited a mass at the hilum in 65 per cent of the cases. A secondary or primary bronchus was involved or occluded in 100 per cent. Complete occlusion occurred in 30 per cent. The primary bronchi were involved in over twice as many cases as in either of the other two types.

In gross these tumors varied from a soft, pink, sarcoma-like mass to hemorrhagic, necrotic and sometimes caseous masses. Hemorrhage and necrosis were prominent and infiltration of the tumors extended along the bronchial tree.

Summary of Classification

A brief description of the histological classification has been given.

Fifty-three and six-tenths per cent of all tumors occurred in the left lung and 46.4 per cent in the right lung. The primary tumors were distributed by lobes as follows: left upper lobe 18.8 per cent, left lower lobe 17.3 per cent, right upper lobe 18.8 per cent, right middle lobe 2.9 per cent, and right lower lobe 8.6 per cent. In 35.1 per cent the primary tumors were located at the hilum and in 4.3 per cent the entire lung was infiltrated from the hilum. The upper lobes and the hilum were the seat of the primary tumor in about an equal number of cases and together constituted the primary location in 72.7 per cent. The infrequency with which the right lower and middle lobes were involved is striking.

Either primary or secondary bronchi were involved or occluded in 68 per cent. In the remaining 32 per cent the bronchi were either negative or not described in relation to the primary tumor mass.

In gross the adenocarcinomas were indistinguishable from the squamous cell tumors unless mucoid secretion was visible. These tumors were usually single, indurated, gray or white masses, situated within the parenchyma of the lung, and involved the larger bronchi in less than one-half of the cases and the hilum in less than one-third. The undifferentiated tumors were soft, hemorrhagic, showed extensive areas of necrosis and, in the great majority of cases, infiltrated along the bronchial tree from a primary site at the hilum.

INCIDENCE

General Incidence: Discussions of the increase in lung carcinoma are so voluminous and numerous that the subject cannot be done full justice here. That more lung carcinomas are being observed, both at autopsy and clinically, is undisputed. However, whether this is an absolute or a relative increase is undecided. Rosahn¹⁷ summarized most of the available autopsy statistics in 1930 and found that lung carcinomas increased 102 per cent in the period from 1920-1928, as compared to the period from 1910-1919, while during these same periods carcinoma in general increased only 30 per cent. He cites this as proof of an absolute increase. Derischanoff¹⁸ found both a relative and an absolute increase and Dissmann¹⁹ believed there was an absolute increase in about the same periods. Sitsen²⁰ found no increase at Innsbruck. Lipschitz²¹ in an analysis of postmortem statistics found an increase in lung carcinoma at Dresden and Zwickau and practically no increase at Copenhagen and Turin, and pointed out that these last two cities are much greater industrial centers than the first two. He believed the incidence of lung carcinoma to be closely related to the residence, vocation and environment of the population. These are only a few of the conflicting reports, and in conclusion it may be said that only when extensive international autopsy statistics, including both urban and rural populations, are available will the question be settled.

The incidence by 5 year periods is given in Table I. Rosahn¹⁷ assembled these statistics at the Boston City Hospital from 1910-1928. Two lung carcinomas occurring between the years 1920 and 1924 have been added and Table I includes the entire period from 1900 to August 1, 1934.

Rosahn found that in the period 1925-1928 the percentage relation of all cases of carcinoma to total autopsies increased 20 per cent, while the percentage relation of primary lung carcinomas to all carcinomas rose 49 per cent, and concluded that this indicated an absolute increase in incidence. The figures previous to 1924 include so few primary lung carcinomas that they are worthy of consideration only in the aggregate. During this 25 year period the per cent of carcinomas occurring at autopsy rose from 6.12 per cent to 10.34 per cent (an increase of 68 per cent) and averaged 7.75 per cent. During this same period lung carcinoma increased in the per cent of

all autopsies but remained fairly constant at an average of 5.71 per cent of all carcinomas. Using these average figures for a basis, in the next 5 years the per cent of carcinoma rose from 7.75 per cent to 11.87 per cent (an increase of 53 per cent) while the per cent of lung carcinoma to all carcinomas increased from 5.71 per cent to 7.68 per cent (an increase of 34 per cent) or an actual decrease in incidence among all cases of carcinoma autopsied. In the subsequent period up to August 1, 1934, the number of cases of carcinoma autopsied decreased slightly from 11.87 per cent to 11.12 per cent (a decrease of 7 per cent) and the per cent of carcinoma of the lung

TABLE I

Incidence of Primary Carcinoma of the Lung at the Boston City Hospital

Year	No. of adult autopsies	Carcinomas		Primary lung carcinomas		
		Total	Autopsies	Total	Autopsies	All carcinomas
			<i>per cent</i>		<i>per cent</i>	<i>per cent</i>
1900-04	931	57	6.12	2	0.21	3.50
1905-09	865	52	6.01	4	0.46	7.69
1910-14	438	34	7.76	2	0.45	5.88
1915-19	526	45	8.55	2	0.38	4.44
1920-24	957	99	10.34	7	0.73	7.07
1925-29	1,532	182	11.87	14	0.91	7.68
1930-34* ...	2,624	293	11.16	38	1.44	12.96
Total	7,873	762	9.67	69	0.87	9.05

* To August 1, 1934

in relation to all carcinomas increased from 7.68 per cent to 12.96 per cent (an increase of 68 per cent). Thus, using comparable periods, there is an absolute increase only in the period from 1930 to August 1, 1934. When traced through single years, this rise has been fairly gradual while the per cent of autopsies revealing carcinoma has remained practically constant. The percentage relation of all cases of lung carcinoma to all carcinomas from 1929 to August 1, 1934 is as follows: 1929, 7.5 per cent; 1930, 10.5 per cent; 1931, 12.7 per cent; 1932, 14.2 per cent; 1933, 10.8 per cent; 1934, 19.1 per cent.

An explanation of this abrupt increase is difficult. However, the tremendous expansion of this hospital in recent years and the increased interest in pulmonary surgery has possibly allowed many

patients to remain in the hospital until autopsied, whereas formerly they were sent to institutions or homes for the care of incurables. No single carcinogenic factor was found at autopsy to explain this rise.

Incidence by Sex: Of the 69 cases examined, 79.7 per cent occurred in males and 20.3 per cent occurred in females, or in the ratio of 1 female to 4.5 males. This is in agreement with most of the statistics consulted. However, of the ten lung carcinomas occurring previous to 1920, five were in males and five in females, or in the ratio of 1:1. From 1920 to August 1, 1934, nine carcinomas

TABLE II

Distribution by Age of Primary Carcinoma of the Lung

Age incidence	Squamous cell carcinoma	Adenocarcinoma	Undifferentiated carcinoma	All tumors
<i>years</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
20-29	0	12	0	2.9
30-39	10	6	9	8.7
40-49	13	23	17	17.3
50-59	34	23	48	36.2
60-69	20	23	17	20.3
70-79	20	6	4	11.5
80-89	0	6	4	2.9
	<i>yrs.</i>	<i>yrs.</i>	<i>yrs.</i>	<i>yrs.</i>
Youngest	36	29	31	29
Oldest	79	89	80	89
Average	57	53.1	53.8	53.7

occurred in females and fifty in males, or in the ratio of 1: 5.5. European investigators have noted this increasing predominance of lung carcinoma in the male and have attempted to explain it on the greater exposure of males to war gasses, and industrial smoke and dust hazards, which probably exerted their irritating influences previous to 1920. It will be interesting to note if the influx of women into industry since the war will result in an increased incidence of lung carcinoma in the female.

Incidence by Age: Brunn ⁶ found that 62 per cent of 576 cases of lung carcinoma occurred in patients between 40 and 60 years of age. Weller ⁵ found the most frequent age was in the 6th and 7th decades, although rarer cases occurred at the extremes of life. Adler ¹ noted

that the 6th decade was the most common period in which patients died of lung carcinoma. In general, the age incidence of carcinoma of the lung coincides with that of all carcinoma.

In Table II the age incidence is given by decades. Thirty-six and two-tenths per cent of all lung carcinomas occurred in the 6th decade and 73.8 per cent occurred in the 5th, 6th and 7th decades. Squamous cell and undifferentiated carcinomas occurred in these same periods. However, adenocarcinomas tended to be slightly more diffusely distributed.

Both the oldest and youngest cases occurred in the adenocarcinomatous group. Ewing²² mentions an adenocarcinoma occurring in a girl aged 18. Pekelis²³ observed 5 cases occurring between the ages of 37-38 and 4 of these were of the adenocarcinomatous type. It would seem that lung carcinoma occurring in the younger age groups is more likely to be of the adenocarcinomatous variety.

METASTASIS

Weller⁵ states that carcinoma of the lung rarely fails to produce metastases. According to Klotz,²⁴ Adler,¹ Rogers¹² and Brunn,⁶ metastases occur most commonly in the regional nodes and involve the liver, skeleton, brain, kidneys, adrenals and pancreas in approximately the order named. Metastases have been recorded in practically every portion of the body. No authors have tabulated metastases of a large series of cases according to the histological structure of the tumors. Also, direct extensions are mentioned so casually that it is doubtful if these have been carefully separated from metastases.

In the present series the body was examined in 67 cases and percentages of somatic metastases have been calculated on this basis in Table III. In 22 cases the head was examined and the intracranial metastases are discussed in a separate table. The tumors have been divided into the three groups mentioned previously and further subdivision is made where it is significant.

Squamous Cell Carcinomas: Twenty-seven cases in this group were examined and of this number 11 per cent failed to metastasize and 18 per cent produced secondary tumors only in the regional nodes.

A separate tabulation of primary tumors with and without cornification was made. Metastases occurred in twenty-one locations from tumors showing cornification and in twenty-four locations from those without cornification. Correlation by per cent of organs involved revealed no significant differences between these groups and it is suggested that these types have approximately the same metastatic potentialities. The presence of epithelial "pearls" and cornification possibly signifies a longer duration of the primary tumor.

Considering the entire group, metastases were most frequently found in the regional nodes. The liver, adrenals, kidneys, pleura, mesenteric nodes, heart and opposite lung were involved in that order of frequency. Rarer metastases occurred as tabulated in Table III.

Adenocarcinomas: Seventeen cases in this group were examined for metastases. Twelve per cent showed no metastases, 6 per cent metastasized only to the regional nodes and 88 per cent metastasized to twenty-two locations.

A tabulation of these tumors based on the presence of mucus revealed that all mucinous carcinomas showed metastases and in no instance were they confined to the regional nodes, while of the non-mucinous carcinomas only 75 per cent metastasized and in 12 per cent only the regional nodes were involved. The liver was involved in 44 per cent of the mucinous type and in only 12 per cent of the non-mucinous. The vertebrae were involved with the same relative frequency. It is concluded that non-mucinous carcinomas are less prone to metastasize than those with mucous secretion.

The adenocarcinomas exhibited a slightly less vigorous tendency to metastasize than the squamous cell tumors. Metastases occurred most frequently in the regional nodes, vertebrae, adrenals and mesenteric nodes in that order. Rarer metastases have been tabulated. The frequency with which bone is involved will be discussed under skeletal metastases.

Undifferentiated Carcinomas: Twenty-three cases in this group were examined and 96 per cent of these tumors showed metastases to thirty-five locations and 4 per cent showed involvement of the regional nodes alone.

These tumors exhibited the most vigorous metastatic powers of any group and spread widely outside of the thorax. Metastases

TABLE III

Distribution of Metastases in Primary Carcinoma of the Lung

Location	Squamous cell carcinoma	Adeno- carcinoma	Undiffer- entiated carcinoma	All tumors
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Peribronchial nodes	59	52	69	61.1
Liver	33	29	43	35.8
Tracheal nodes	18	23	24	25.3
Adrenals	33	17	21	25.3
Vertebrae	7	29	28	20.8
Kidneys	30	6	16	19.6
Retroperitoneal nodes	7	6	39	17.9
Mesenteric nodes	22	17	21	17.9
Cervical nodes	11	12	16	13.4
Opposite lung	14	12	4	10.4
Mediastinal nodes	0	0	16	10.4
Stomach	11	6	8	9.1
Heart	18	0	4	9.1
Pancreas	0	6	21	9.1
Iliac nodes	11	0	8	7.4
Ribs	11	12	0	7.4
Left pleura	14	0	0	5.9
Right pleura	11	6	0	5.9
Axillary nodes	0	6	12	5.9
Skin	4	6	8	5.9
Ileum	7	0	4	4.4
Spleen	4	0	8	4.4
Terminal phalanges	0	0	8	2.9
Spinal meninges	0	0	8	2.9
Uterus	4	6	0	2.9
Cecum	0	0	8	2.9
Esophagus	0	6	4	2.9
Femur	0	6	4	2.9
Diaphragm	7	0	0	2.9
Clavicle	0	6	0	1.5
Peritoneum	0	0	4	1.5
Jejunum	0	0	4	1.5
Tibia	0	0	4	1.5
Ilium	0	0	4	1.5
Appendix	0	0	4	1.5
Inguinal nodes	4	0	0	1.5
Gall-bladder	0	0	4	1.5
Psoas muscle	4	0	0	1.5
Testes	4	0	0	1.5
Ureter	4	0	0	1.5
Ovary	0	6	0	1.5
Broad ligament	0	6	0	1.5
Inferior vena cava	4	0	0	1.5
Pericardium	0	0	4	1.5
Radius	0	0	4	1.5

have been tabulated in Table III and it is of special interest to note that in 8 per cent there were extradural metastases in the spinal canal with compression of the cord. In an additional 8 per cent the terminal phalanges were involved. These odd metastases indicate the tendencies of this group to grow into blood vessels and produce secondary tumors which closely resemble sarcomas when biopsied.

Skeletal Metastases: Secondary tumors occurred in bone in 28.3 per cent of cases. Of these tumors involving bone, 31 per cent were adenocarcinomas, 42 per cent undifferentiated and 26 per cent squamous cell carcinomas. Fifty-five per cent of all mucinous adeno-

TABLE IV

Intracranial Metastases of Primary Carcinoma of the Lung

	Squamous cell carcinoma	Adeno- carcinoma	Undiffer- entiated carcinoma	Total
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Cerebrum	22	33	28	22.7
Cerebellum	22	0	28	18.1
Dura	11	0	0	4.5
Pons	11	0	0	4.5
Pituitary	11	0	0	4.5

carcinomas metastasized to bone and only 12 per cent of the non-mucinous type. The relatively high frequency with which adenocarcinomas, and especially the mucinous type, involve bone is striking.

In all, 19 cases showed metastases with involvement of the vertebrae in 56 per cent, ribs in 21 per cent, skull, femur, terminal phalanges in 8 per cent each, and ilium, tibia and clavicle in 4 per cent each.

Routine examination of the lumbar spine and ribs was made in the majority of instances. However, other portions of the skeleton were not examined unless superficial examination aroused suspicion. Of necessity, these figures are incomplete.

Intracranial Metastases: The intracranial contents were examined in 22 cases and metastases were found in 36.3 per cent. Metastases were found with the following frequency: 44 per cent of 9 squamous cell carcinomas, 33 per cent of 6 adenocarcinomas, and 28 per cent of 7 undifferentiated carcinomas.

Table IV demonstrates that the cerebrum and cerebellum are the most frequent sites of metastases. The frequency with which secondary growths from primary lung carcinoma occur in the cranial cavity has been stressed repeatedly. Fried⁸ found that 31 per cent of 47 cases metastasized intracranially and in many instances they were operated upon for primary intracranial tumors, and he emphasized the importance of eliminating the possibility of metastatic tumor of the lungs in all cases of suspected intracranial newgrowths.

Summary of Metastases

Somatic metastases occurred in 92 per cent of 67 primary lung carcinomas examined and involved forty-five locations. The regional-nodes, liver, adrenals, vertebrae, kidneys, retroperitoneal nodes, mesenteric nodes and cervical nodes, opposite lung, mediastinal nodes and stomach are the sites most frequently involved and the frequency is in the order named. Rarer metastases were numerous and widespread, as has been enumerated.

Skeletal metastases occurred in 28.3 per cent.

Cerebral metastases occurred in 36.3 per cent of 22 cases examined.

The undifferentiated, small cell type showed the most vigorous tendency to metastasize and the squamous cell, mucinous and non-mucinous adenocarcinomas follow in the order named.

From a study of metastatic lesions it seems evident that these tumors metastasize most frequently by the lymph channels, and commonly by the blood stream. No positive proof of metastasis by the air passages was found.

EXTENSIONS

Direct tumor extension involving vital structures is frequently mentioned in the literature. The heart and pericardium have been mentioned as direct sites of extension. A few instances of direct proliferation to the superior vena cava or to the regional nerves with resulting pain or dysphagia have been noted. For the most part, however, it is difficult to separate metastases from extensions in the larger series in which anatomical studies have been made. In the present series a careful attempt has been made to separate these two manifestations of malignancy and they will be discussed below according to the types of tumors.

Squamous Cell Carcinomas: In 27 cases examined 55 per cent of primary tumors showed direct regional extension. In 14 per cent there were extensions without metastases. Cornifying primary tumors extended in 50 per cent and non-cornifying in 57 per cent. Structures involved were the mediastinum, pericardium, heart, aorta, right pleura, left pleura, esophagus, diaphragm and ribs. In 1 case the superior vena cava was invaded with thrombosis of that vessel. In another case the primary tumor directly proliferated

TABLE V

Distribution of Extensions in Primary Carcinoma of the Lung

Location	Squamous cell carcinoma	Adeno- carcinoma	Undiffer- entiated carcinoma	All tumors
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Mediastinum	33	17	52	35.8
Pericardium	30	11	39	28.3
Aorta	18	6	23	17.7
Left pleura	11	0	34	16.4
Heart	18	0	17	13.4
Right pleura	14	11	8	11.9
Esophagus	11	0	17	10.4
Ribs	7	17	4	8.9
Diaphragm	11	6	8	8.9
Superior vena cava	4	11	4	7.4
Cervical region	4	6	0	2.9
Liver	4	0	0	1.5
Adrenal	4	0	0	1.5
Opposite lung	0	0	4	1.5
Skin	4	0	0	1.5
Spinal meninges	0	0	4	1.5
Clavicle	0	0	4	1.5

through the diaphragm and involved the liver and right adrenal. In another case the cervical region and skin were directly invaded.

Adenocarcinomas: In 17 cases examined 46 per cent extended from the primary site. In no instances were extensions present without metastases. Fifty-five per cent of the mucinous type extended locally and 37 per cent of the non-mucinous. Structures involved were the mediastinum, ribs, right pleura, superior vena cava, left pleura, diaphragm and cervical region. In 2 cases there was thrombosis of the superior vena cava as a result of tumor extension.

Undifferentiated Carcinomas: Of the 23 cases examined in this group 65 per cent involved the mediastinum or thoracic structures.

In 4 per cent there was extension without metastases. Extensions have been tabulated in Table V. Of special interest is one tumor which extended posteriorly between the ribs and compressed the spinal cord at the level of the fifth thoracic vertebra. In another instance the superior vena was thrombosed as a result of tumor extension.

Summary of Extensions

Of 67 cases examined 56.7 per cent showed extensions. In 6 per cent there were extensions without metastases. Sixty-five per cent of undifferentiated carcinomas, 55 per cent of squamous cell tumors and 46 per cent of all adenocarcinomas, extended locally. Only 37 per cent of non-mucinous adenocarcinomas extended locally. The percentage with which various structures were involved has been tabulated. Thrombosis of the superior vena cava occurred in 4 cases and in 2 cases the tumors extended to the cervical region and in 1 case the spinal canal was invaded with compression of the spinal cord.

Undifferentiated carcinomas exhibited the most marked tendency to extend locally and squamous cell, mucinous and non-mucinous carcinoma follow in the order named.

ASSOCIATED PATHOLOGICAL FINDINGS

Evidence of associated pulmonary inflammatory conditions were found in 58.8 per cent of cases. Either an active or organizing bronchopneumonia was present in 27.8 per cent; bronchiectasis, usually with small bronchiectatic abscesses, was found in 17.6 per cent. Four and four-tenths per cent showed lobar pneumonia, and fibrinous pleuritis and pneumoconiosis were present in 1.5 per cent each.

Complete atelectasis of a lobe or a lung occurred in 20.5 per cent and pulmonary infarction in 4.4 per cent. These conditions may be interpreted as the result of the mechanical occlusion of a bronchus or ramification of the pulmonary artery by direct tumor proliferation.

Chronic inflammatory disease of the lungs has frequently been accused of producing a predisposition to pulmonary cancer. It is believed that the repeated destruction of the epithelial cells lining

the bronchi leads ultimately to metaplasia and the production of atypical cells and these are thought to be pre-malignant. However, the great frequency with which the primary tumors are located at the hilum or in the upper lobes (72.7 per cent), and the frequency with which bronchiectasis, pulmonary abscesses and chronic pneumonia involve the lower lobes appears to preclude the presumption that these latter processes are sources of chronic irritation and the underlying cause of lung carcinoma in a significant number of cases. In fact, the right lower lobe, considered the most frequent location of bronchiectasis and pulmonary abscesses, was the primary site of lung carcinoma in only 8.6 per cent of cases and one of the least frequently involved lobes. In the majority of instances where bronchiectasis or chronic pneumonia was found, they occurred distal to the tumor mass and the natural assumption was that they were secondary manifestations of the tumor resulting from blockage of lymphatics and bronchi.

Active tuberculosis was present in only 1.6 per cent and healed parenchymal tuberculosis in 5.9 per cent. Thus, the total of 7.4 per cent is slightly less than that reported by Kikuth (quoted from Weller ⁵) of 8.7 per cent, and is roughly in accord with the known incidence of tuberculosis in unselected cases. "Kikuth felt that tuberculosis plays a small rôle, if any, in determining a malignant pulmonary condition, occupying in this respect exactly the same position as a considerable number of other chronic inflammatory diseases."

Schmorl felt that the inhalation of dust and the presence of pneumoconiosis was of significance, especially in the production of lung carcinoma in the Schneeberg miners. Certainly, the frequency with which these conditions are associated at Schneeberg is more than coincidental and eventually a specific etiological factor may explain these cases. However, the relative infrequency with which these two conditions are associated elsewhere and in other mines lends doubt to the theory that the mechanical irritation is an etiological factor and it would seem more likely that the answer rests in some specific quality of the Schneeberg ore. In the present series of cases pneumoconiosis was found in only 2.9 per cent of instances, or in 2 cases, and in both of these the fibrosis was only of moderate degree. It would appear that pneumoconiosis is of insignificant importance in the production of lung carcinoma in general.

Associated extrapulmonary pathological findings were distributed as follows: pyelonephritis 13.4 per cent, cholecystitis and endocarditis 4.4 per cent each, pericarditis, acute pancreatitis and alcoholic cirrhosis 2.9 per cent each, mitral stenosis, chronic glomerular nephritis, peptic ulcer, diverticulitis, peritonitis and esophageal obstruction in 1.5 per cent each. These findings are not inconsistent with postmortem findings in unselected cases of an older age group predominantly male.

PLEURAL CAVITIES

Pleural effusion may result either from the inflammatory processes commonly associated with primary lung carcinoma or as a result of tumor implants on the pleural surfaces. The pleural cavities were involved by tumor in 39.3 per cent of cases, either by extension or metastasis. In 11 per cent there was fluid in both pleural cavities and in 32 per cent fluid was present only on the same side as the primary lung carcinoma. In 6 per cent fluid was present on the opposite side and in 50 per cent no fluid was present. Most frequently the fluid was described as serosanguineous; however, in many instances it was clear, colorless or yellow fluid. In 2.9 per cent of cases it was seropurulent and a definite fibrinous pleuritis was present, and in 1.5 per cent frank empyema existed as a result of a bronchial fistula. These latter conditions were found on the same side as the primary tumor.

Fibrous adhesions, evidence of healed pleuritis, occurred on both sides in 38 per cent; on the same side as the primary tumor only, in 54 per cent; and on the opposite side only, in 1.5 per cent. The pleural cavities were negative in 10 per cent of cases.

Adenocarcinomas showed a slightly greater tendency to involve the pleura with the accumulation of fluid and the production of adhesions than either of the other two types described.

SUMMARY AND CONCLUSIONS

1. Sixty-nine cases of primary carcinoma of the lung, verified at autopsy, have been presented and divided into three groups, namely: (a) squamous cell carcinoma, (b) adenocarcinoma, (c) undifferentiated carcinoma.

2. Squamous cell carcinomas constituted the largest single group and 42 per cent of the entire series. The left lung and upper lobes were the most common site of the primary tumor, and 61 per cent involved a bronchus. Cavitation in the primary tumor occurred in 17 per cent. Metastases and extensions were not so widespread as in the undifferentiated group, but were more extensive than in the adenocarcinoma.

3. Adenocarcinomas constituted 24 per cent of the series and were composed of 53 per cent mucinous carcinoma and 47 per cent non-mucinous carcinoma. These tumors probably all originated from the epithelium lining the bronchi or from the peribronchial mucous glands. The mucinous type frequently metastasized and occasionally extended, but appeared less malignant than the non-mucinous group. They involved bone more frequently than any other type.

Non-mucinous carcinomas were the least malignant and were occasionally confined to a lobe or a lung. They frequently involved the pleura.

4. Undifferentiated carcinomas constituted 33 per cent of this series. Primary tumors occurred slightly more frequently in the left lung, always involved a bronchus and occasionally infiltrated an entire lung. This group showed the most vigorous tendency to metastasize widely and to extend locally.

5. All lung carcinomas in this series occurred most frequently in the left lung, in the upper lobes and at the hilum. Primary tumors were a single mass in 95.7 per cent of the cases and usually involved or occluded a bronchus. These carcinomas metastasized widely and primary tumors were very prone to extend regionally. Skeletal and intracranial metastases were common.

6. An absolute increase in the general incidence of lung carcinoma occurred at the Boston City Hospital in the period 1930 to August 1, 1934, and is possibly explainable as a selective phenomenon.

7. Males were affected predominantly and in the ratio of 1 female to 4.5 males. The incidence in males has increased in the past 15 years.

8. The majority of cases occurred in the 6th and 7th decades. Adenocarcinomas tended to occur more frequently at the extremes of life.

9. Associated pulmonary inflammatory conditions occurred in 58.8 per cent of the cases.

10. The incidence of pulmonary tuberculosis and pneumoconiosis in this series was consistent with the incidence in unselected cases.

NOTE: I wish to express my appreciation to Dr. F. Parker, Jr., for helpful criticism and aid in preparing this paper, and to Dr. J. H. Peers for aid in the classification of this series of tumors.

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HISTOLOGICAL EFFECTS OF POTASSIUM IODIDE AND THYROID SUBSTANCE ON THE THYROID GLAND OF THE GUINEA PIG IN EXPERIMENTAL SCURVY *

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INTRODUCTION

1. *The Effect of Potassium Iodide on the Thyroid Gland*

The histological results of potassium iodide feeding on the normal thyroid gland of the guinea pig have been recorded by Gray,¹ Gray, Haven and Loeb,² Gray and Loeb,³ Gray and Rabinovitch,⁴ Loeb,⁵⁻⁹ McCordock,¹⁰ Rabinovitch,^{11, 12} Rabinovitch and Gray,¹³ and Silberberg.¹⁴ They found that short periods of either oral or intraperitoneal administrations of potassium iodide (usual dose from 0.01 to 0.1 gm. of potassium iodide) cause a stimulation of the normal thyroid gland. Rabinovitch¹¹ showed a definite relation between the amount of potassium iodide fed and the increase in proliferative activity of the epithelium, as estimated by the number of mitoses. Gray and Loeb,³ and Rabinovitch¹² noted that the action of the potassium iodide on the thyroid gland does not reach its maximum until after 16-18 days, this being the period of marked proliferation in normal guinea pigs.

Gray, Haven and Loeb,² Gray and Loeb,³ Loeb,^{5,7} and Rabinovitch¹⁵ observed an entire cessation in mitotic activity after feeding potassium iodide for a period of 30 days because of the pressure exerted on the cells of the acini as a result of the increase in quantity of the colloid. Loeb¹⁶ states that the colloid produced under potassium iodide stimulation does not leave the acini in a sufficient quantity but remains largely stored up in the gland, and by its injurious pressure on the walls of the acini may lead to a gradual inhibition of the glandular activity. In other words, it is possible that potassium iodide causes a retention of the thyroid hormone within the gland.

* Received for publication November 24, 1934.

2. *The Effect of Thyroid Feeding on the Thyroid*

Structural signs of inhibition in the activity of the thyroid gland, similar to those noted above, have been reported by Gray, Haven and Loeb,² Gray and Loeb,³ and Gray and Rabinovitch¹⁷ after oral administration of thyroid substance to normal guinea pigs. They also noted the number of mitoses to be lower than in the normal controls. According to Gray, Haven and Loeb,² in thyroid feeding an excess of thyroxin in the circulation prevents the mobilization of colloid in the gland, which thus remains solid. In addition, the thyroxin may perhaps gradually cause an atrophy of the epithelium as an expression of its inactivity.

3. *The Thyroid Gland in Experimental Scurvy*

Rondoni and Montagnani¹⁸ observed hemorrhagic lesions of the thyroid gland as being characteristic in scorbutic guinea pigs. McCarrison¹⁹⁻²¹ described a marked enlargement of the thyroid resulting in an increased weight of the gland, sometimes amounting to two or three times the weight of that in the healthy animal. When he examined these glands histologically he found the enlargement to be due mainly to hemorrhagic infiltration of the organ. He concludes that a scorbutic diet of crushed oats and autoclaved milk may cause a considerable enlargement of the thyroid gland in guinea pigs. Bessesen²² also found an irregular enlargement of the thyroid in various stages of experimental scurvy in guinea pigs.

Löwy²³ found no histological changes in the thyroid gland during scurvy, as compared with the gland of normal control guinea pigs. Meyer²⁴ described thyroid glands of scorbutic guinea pigs as having a tendency to show a reduction in the amount of colloid and an increase in the amount of "intrafollicular (desquamated) cells," as he called them. He also noted these elements varied considerably in amount in the same gland, as well as in different thyroid glands. He states that his work is not inclusive enough (insufficient number of cases) to draw reliable conclusions and consequently he believes, along with Löwy, that no "noteworthy changes" take place in the thyroid glands of guinea pigs fed the scurvy-producing diet for 30 days.

Harris and Smith²⁵ studied the changes in the thyroid during chronic scurvy lasting 97 days. They reported a decrease in the

amount and an increase in the vacuolation of the colloid. This was accompanied by an increase in the height of the follicular epithelium and an increase in the number of interfollicular cells. They suggested that vitamin C might function in the regulation of iodine metabolism.

It is known that the thyroid is associated with iodine metabolism. Therefore, it was the purpose of this investigation to determine the histological effects of potassium iodide and thyroid substance on the thyroid gland in experimental scurvy, and to determine whether or not the administration of these iodine compounds would tend to prolong the life of scorbutic animals.

MATERIAL AND TECHNIQUE

Since young guinea pigs are more susceptible to scurvy than adults, only active individuals in good nutritive condition and weighing between 250 and 350 gm. were used. The control and experimental groups were divided equally with regard to sex. All of the experimental animals were kept at an approximately constant temperature. They were kept in individual sanitary cages with open wire-mesh bottoms to allow the excreta to fall through, thus tending to prevent coprophagy and eliminate any source of vitamin C that might occur in this manner. The cages were cleaned thoroughly at regular intervals.

The animals were housed in their respective cages and fed the basal ration plus green food, both *ad libitum*, and also 3 cc. of orange juice daily for a week before the beginning of the experimental period. During this period of observation attention was paid to general activity and willingness to eat the "synthetic" ration. The experiment proper was then begun by discontinuing the green food and continuing the administration of orange juice to the control, starvation, and chronic scurvy animals, as indicated below, but not to the animals on the acute scurvy diet. The animals were divided into groups and fed diets and iodine compounds respectively, as indicated in Table I. The diets used were as follows.

Scurvy-Producing Diet (Basal Diet): This consisted of alfalfa meal and wheat flour, mixed in equal amounts by weight and moistened with water. Whole oats and tap water were supplied *ad libitum*. The ration was prepared freshly every morning and a sufficient amount for one day was placed in low dishes in the cages.

Chronic Scurvy Diet: The basal diet plus 0.5 cc. of orange juice every second day.

Starvation Diet: Water and 1 cc. of orange juice were given daily.

TABLE I

Experimental Procedure

Animal groups	No. animals used	Days on experimental diet	Days on iodine compounds
<i>Controls</i>			
(1) Normal	6	0-110	0
(2) Normal +KI	4	31-111	11-55
(3) Normal +thyroid	2	31-118	31-53
<i>Starvation</i>	4	5-10	0
<i>Acute scurvy</i>			
(1) Iodine-free	2	21-29	0
(2) Iodine compounds from first day			
(a) KI	1	17	17
(b) Thyroid	1	14	14
(3) Iodine compounds after 21 days			
(a) KI	6	22-33	1-12
(b) Thyroid	6	23-31	2-10
<i>Chronic scurvy</i>			
(1) Iodine-free	2	56-126	0
(2) KI after 56 days	5	63-138	7-82
(3) Thyroid after 65 days	4	72-120	7-55
<i>Total scorbutic animals</i>			
(1) Iodine-free	4	21-126	0
(2) Iodine compounds			
(a) KI	12	17-138	1-82
(b) Thyroid	10	14-120	2-55

Normal Diet: The same kind and amount of food substances were given as were used in the scurvy-producing diet with the addition of 3 cc. of fresh orange juice daily, administered orally by pipette, to each guinea pig.

The dose of potassium iodide consisted of 0.01 gm. (Merck's C. P. granular potassium iodide) in 1 cc. of distilled water. A dose of thyroid substance amounted to 0.1 gm. (Lilly's U. S. P. thyroid, 1 gm. representing 5 gm. of fresh thyroid gland) in 1 cc. of distilled

water. These solutions were administered daily by mouth through pipettes.

The animals were weighed at 3 day intervals, except in the starvation experiments, in which they were weighed daily.

In all animals on the scurvy diet typical and usually severe symptoms of scurvy developed. When they had reached the stage of advanced scurvy, and almost at the point of death in the acute scurvy cases, chloroform was administered and both lobes of the thyroid gland were immediately removed and placed in Zenker's fixative. They were embedded in paraffin, sectioned at 7μ thickness in complete serial sections, and stained with Delafield's hematoxylin and eosin. These stains were found to be satisfactory in bringing out both the cytoplasmic and nuclear structures.

The following points were considered in studying the slides: (1) condition of follicles, (2) colloid, (3) epithelium, (4) interfollicular cells, and (5) phagocytes. Sections from approximately the same areas of the thyroid gland were used in these studies.

RESULTS

1. Effects of Various Diets on the Condition of the Animals

The weight curves obtained were typical and characteristic of the diets on which the animals had been placed. They were similar to those given by Hess.²⁶

The animals receiving 3 cc. of orange juice daily appeared to be in good health and active throughout the entire experimental period. Those on the starvation diet did not exhibit any symptoms of scurvy. The animals fed only the basal diet lived from 14 to 33 days, with an average of 28.2 days, on account of the development of acute scurvy. However, the animals that received 0.5 cc. of orange juice every second day, in addition to the regular basal diet, exhibited chronic paralysis, soreness to touch, fragility of bones, decreased consumption of food with loss of weight, and lived from 56 to 138 days, with an average of 97.7 days. In some cases there was no paralysis of the limbs, even though other symptoms developed. In many instances the teeth were broken off, but this was never seen in normal controls. The histological results are presented in tabular form in Table II.

2. *The Normal Thyroid Gland*

In the normal thyroid gland, as a general rule, the follicles are rounded, regular and medium in number and size. The epithelium appears in most cases to be cuboidal, with an average or medium height and with round nuclei, as shown in Figure 1. Phagocytes which are located in the colloid are few in number. There is considerable variation in the quantity of colloid of the normal thyroid, as revealed by a comparative study of the various glands. It is uniformly stained but varies considerably in vacuolations from practically solid (Fig. 1) to extreme vacuolations throughout. However, this extreme vacuolation is probably exceptional, being observed in only 1 case. At times the colloid fills the follicles completely, whereas in other cases it is greatly retracted from the follicular wall. This condition may be due to shrinkage effects produced during preparation of the material. The latter two conditions may occur in the same gland, but usually one condition alone is found throughout.

Starvation from 5 to 10 days had practically no effect on the thyroid gland, as compared with the normal, since they are very similar in most respects. The most noticeable difference is that the colloid is not as uniformly stained as in the normal.

3. *The Effect of Potassium Iodide on the Thyroid Gland*

Potassium iodide causes the follicles to become irregular in shape, and larger in size, as seen in Figure 2. There is a decrease in number of follicles since they unite with one another (Fig. 5). The colloid is not uniformly stained and is usually retracted slightly from the follicular wall. In short periods of administration (14 days) the colloid is soft, always peripherally vacuolated, and frequently honey-combed throughout, but not increased in amount. The epithelium is slightly higher and phagocytes are numerous. However, there is a characteristic change during longer periods of administration (55 days), as noted in Figure 2. The colloid becomes harder, more solid, less vacuolated and more abundant. The epithelium, because of the pressure exerted on it by the colloid, becomes low, flat, thin and rectangular, with flattened nuclei. Many cases are seen where the thinness results in a break in the follicular wall and a consequent

TABLE II

Experimental Results

Animal groups	Microscopic findings			
	Follicles	Colloid	Epithelium	Interfollicular cells
<i>Controls</i>				
(1) Normal	Rounded, regular, medium size and number	Uniformly stained, peripherally vacuolated	Cuboidal, average height, with round nuclei	Average number
(2) Normal +KI	Irregular, slightly increased in size	Slightly increased, non-uniformly stained, peripherally vacuolated, frequently honeycombed	Low, flat, thin, rectangular with flattened nuclei	Decreased number
(3) Normal +thyroid	Numerous, small, irregular	Non-uniformly stained, non-vacuolated	Cuboidal, medium height, with round nuclei	Slightly increased
<i>Starvation</i>	Same as control	Non-uniformly stained, otherwise like control	Same as control	Same as control
<i>Acute scurvy</i>				
(1) Iodine-free	Irregular, reduced in number	Non-uniformly stained, reduced, peripherally vacuolated, sometimes honeycombed	Very high, elongated, nuclei round	Increased
(2) (a) KI after 21 days	Small, rounded, average number	Non-uniformly stained, slightly vacuolated peripherally, sometimes honeycombed. Average amount	Tends to be low, flat, thin, with flattened nuclei	Average number
(2) (b) Thyroid after 21 days	Small, irregular, average number	Non-uniformly stained, solid, average amount	High, elongated, with round nuclei	Average number
<i>Chronic scurvy</i>				
(1) Iodine-free	Small, irregular, slightly below average number	Decreased, non-uniformly stained, peripherally vacuolated, sometimes honeycombed	High, elongated with round nuclei	Increased
(2) KI after 56 days	Average number and size, irregular	Non-uniformly stained, average amount, solid, increased in long periods of feeding	Low, flat, rectangular with flattened nuclei, breaking through in many places	Reduced
(3) Thyroid after 65 days	Average size and number, irregular	Non-uniformly stained, sometimes vacuolated, slightly increased in long periods of feeding	High, elongated, with round nuclei	Slight increase

coalescence of the colloid contained within (Fig. 5). There is a reduction in number of the interfollicular cells, and phagocytes are not so prevalent, being represented as degenerated structures appearing as dark spots in the colloid (Fig. 2).

4. The Effect of Thyroid Feeding on the Thyroid Gland

Thyroid substance also causes the follicles to become irregular and small, but more numerous as shown in Figure 3. The colloid is non-uniformly stained, retracted greatly from the follicular wall, average in amount and non-vacuolated. The epithelium in most cases is cuboidal, of medium height with round nuclei. There is a slight increase in the number of interfollicular cells and phagocytes are rare.

5. The Thyroid Gland in Experimental Scurvy

(A) *The Thyroid in Chronic and Acute Scurvy:* Chronic scurvy of 126 days duration, without the addition of any iodine compounds, causes more marked changes in the thyroid (Fig. 4) than acute scurvy. The follicles have a higher epithelium and are more irregular and reduced in number. The non-uniformly stained colloid is more vacuolated peripherally and sometimes throughout, and further reduced in amount, tending to disappear entirely from many of the follicles. There is a tendency for the epithelium, which is high with round nuclei, to become columnar. The interfollicular cells are increased in number but the phagocytes are very scarce. These changes are not so marked in chronic scurvy lasting for 56 days. Likewise, these conditions are more marked in animals fed the basal diet alone for 29 days than in those on the same diet for 21 days.

(B) *The Effect of Potassium Iodide in Experimental Scurvy:* Potassium iodide administered to scurvy guinea pigs produced similar changes in the thyroid (Fig. 5) as described above for the normal gland (Fig. 2). The histological changes were not as noticeable in short periods of administration, in either acute or chronic scurvy, as they were in longer periods.

(C) *The Effect of Thyroid Feeding on the Thyroid Gland in Experimental Scurvy:* The administration of thyroid substance to scurvy guinea pigs produces the same changes in the thyroid glands as has previously been described for the controls (Fig. 3). Thyroid glands

in the chronic scurvy condition, to which thyroid substance has been administered for a short time (7-11 days), resemble those of 126 day chronic scurvy (Fig. 4), whereas those in which the administration lasted much longer have a different appearance, as noted in Figure 6. Long periods of thyroid administration cause the gland to return to a condition similar to that of the normal. In other words, it appears that potassium iodide and thyroid substance offset the action of scurvy on the thyroid gland, causing a decided change in its histological appearance.

Observations were made to determine whether or not potassium iodide and thyroid substance would prolong the life of the animal in experimental scurvy. Animals on the basal diet plus potassium iodide had an average life of 28 days, whereas those on the same diet, with the addition of thyroid substance, lived on the average 28.5 days. Thus, in the cases studied it was noted that these iodine compounds did not tend to prolong the life of the individuals, since Sherman and Smith²⁷ found the survival period of scorbutic animals to vary from 26 to 34 days.

DISCUSSION

The normal thyroid glands described above compare very favorably with those described by Loeb,⁵⁻⁹ Gray,¹ Rabinovitch,^{11,12} McCordock,¹⁰ and others. One exception to the general conditions was observed in which there was an excessive amount of vacuolation.

The glands from animals on a starvation diet (5-10 days) appeared like those of the controls in every way except that a difference in the staining power of the colloid was noted. Rondoni and Montagnani,¹⁸ Löwy,²³ and Harris and Smith,²⁵ likewise found no significant histological changes in the structure of the thyroid gland in guinea pigs as a result of starvation.

The histological changes occurring in the thyroids of guinea pigs with experimental chronic scurvy are similar to those previously described by Harris and Smith²⁵ in chronic scurvy of 97 days duration. They likewise found similar changes, but less marked, in acute scurvy. Hemorrhagic infiltrations, as observed by McCarrison,²⁰ and Rondoni and Montagnani,¹⁸ were seen to a slight extent in the normal animals, but were more noticeable in acute scurvy of 29 days and considerably greater in chronic scurvy of 126 days duration.

Whether they are extensive and great enough to cause the increased weight observed by McCarrison is not known.

The histological results of potassium iodide feeding reported in the present work (Fig. 2) agree with those of other workers. Furthermore, it seems that potassium iodide has the same action on the thyroid gland in acute and chronic scurvy (Fig. 5) as it does on a normal gland (Fig. 2), when administered for a corresponding period of time. That is, potassium iodide seems to offset the action of scurvy on the thyroid, and instead induces its own characteristic effects. The idea of Loeb¹⁶ that potassium iodide causes a retention of the thyroid hormone within the gland, might serve as an explanation for the increase of colloid observed, and the failure to observe the histological changes characteristic of scurvy, as described by Harris and Smith,²⁵ and the writer.

It was found that administration of thyroid substance to normal guinea pigs causes morphological indications of inhibition in the activity of the thyroid gland, as reported by earlier investigators. The hypothesis of Gray, Haven and Loeb² that the increased amount of thyroxin in the circulation due to thyroid feeding does not allow the mobilization of colloid in the gland, might explain why the colloid, in the case of feeding thyroid substance to chronic scurvy guinea pigs, does not become vacuolated and reduced, as it does in iodine-free chronic scurvy. The administration of thyroid substance in acute and chronic scurvy (Fig. 6), causes a definite and decided change toward the condition in the normal gland during thyroid feeding. This change is more pronounced in the longer periods of administration.

The fact that the administration of iodine compounds seemed to cause the thyroid to approach its normal condition in the absence of vitamin C, without prolonging the life of guinea pigs in experimental scurvy, seems to indicate that vitamin C is not concerned with iodine metabolism, as suggested by Harris and Smith.²⁵

SUMMARY AND CONCLUSIONS

1. The thyroid gland in scurvy presents irregular follicles with higher epithelium, a reduced amount of non-uniformly stained but extensively vacuolated colloid, and an increase in the interfollicular cells. These changes are more marked in chronic scurvy of long duration than in acute scurvy.

2. Potassium iodide, when administered to animals with scurvy, causes a decrease in the number of vacuoles and an increase in amount of the colloid, accompanied by a flattening of the epithelium and a decrease of the interfollicular cells. Thyroid substance produces similar results except that the epithelium is not flattened but is returned to the normal medium height.

3. Potassium iodide and thyroid substance, in the doses administered, do not tend to prolong the life of the animal in experimental scurvy. Thus, it appears that vitamin C is not concerned with iodine metabolism.

NOTE: The writer wishes to express his deepest appreciation to Dr. D. Ludwig for his advice and careful criticism throughout the course of this investigation.

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DESCRIPTION OF PLATES

PLATE 63

- FIG. 1. Photomicrograph of a section of a normal gland. Female, on diet 21 days. The follicles are rounded, regular, and medium in size and number. The epithelium is cuboidal, of average or medium height with round nuclei. The interfollicular cells are average in number. There are a few phagocytes. The colloid is practically solid but slightly retracted. $\times 800$.
- FIG. 2. Photomicrograph of a section of thyroid gland of a female guinea pig fed a normal diet for 56 days before the administration of potassium iodide. Potassium iodide then given for 55 days. Decreased number of irregular follicles. Colloid less vacuolated and more abundant. Low, flat, thin epithelium with flattened nuclei. Reduction of interfollicular cells. Dark spots in the colloid represent degenerated phagocytes. $\times 800$.
- FIG. 3. Photomicrograph of a section of thyroid gland of a male guinea pig fed a normal diet for 53 days before the administration of thyroid substance. Thyroid substance then given for 65 days. Irregular follicles. Colloid greatly retracted but average in amount and non-vacuolated. Cuboidal epithelium. Degenerated phagocytes are rare. $\times 800$.

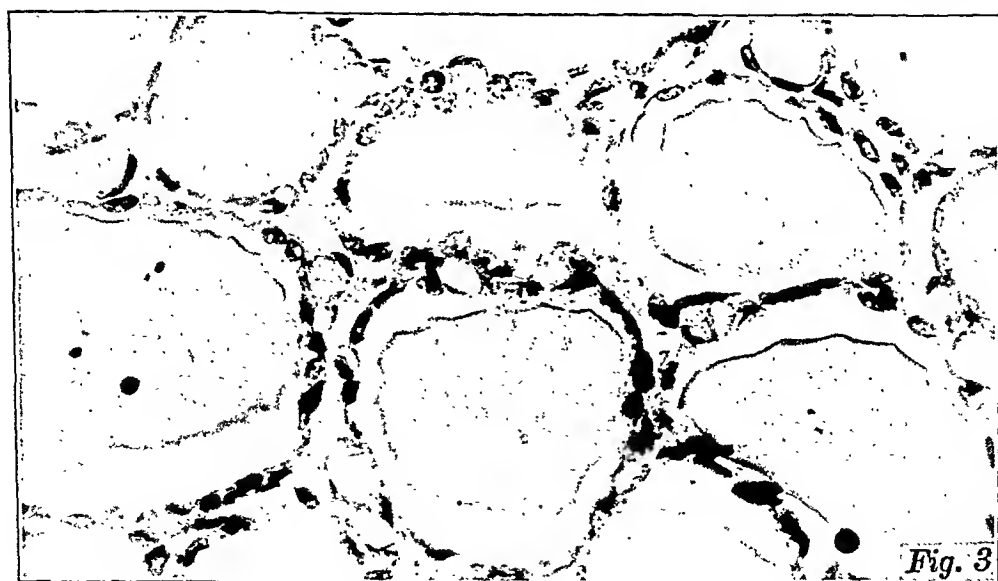
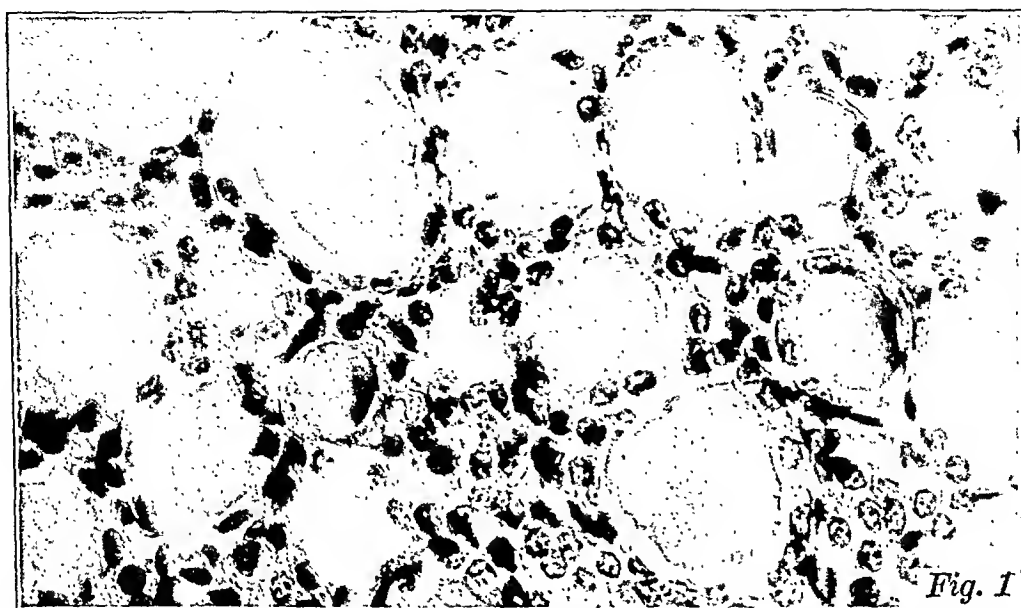
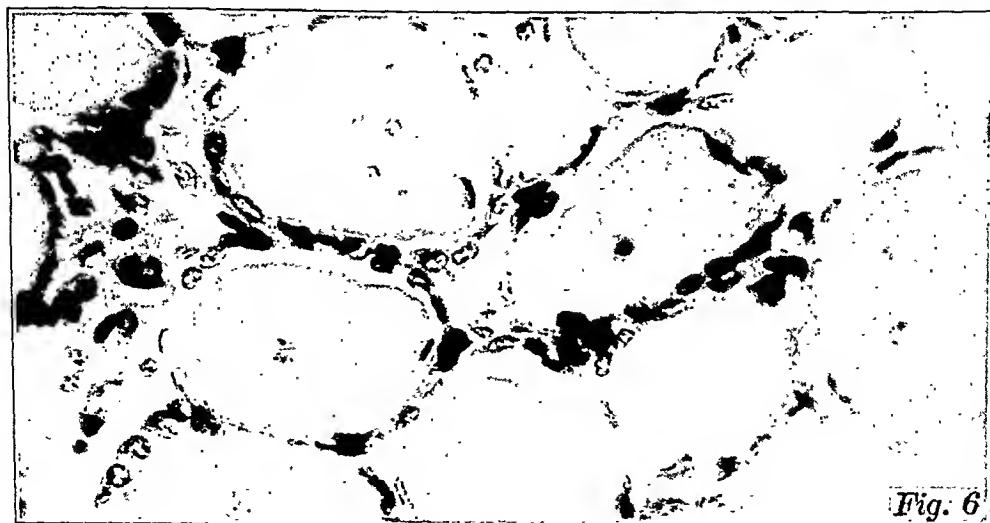
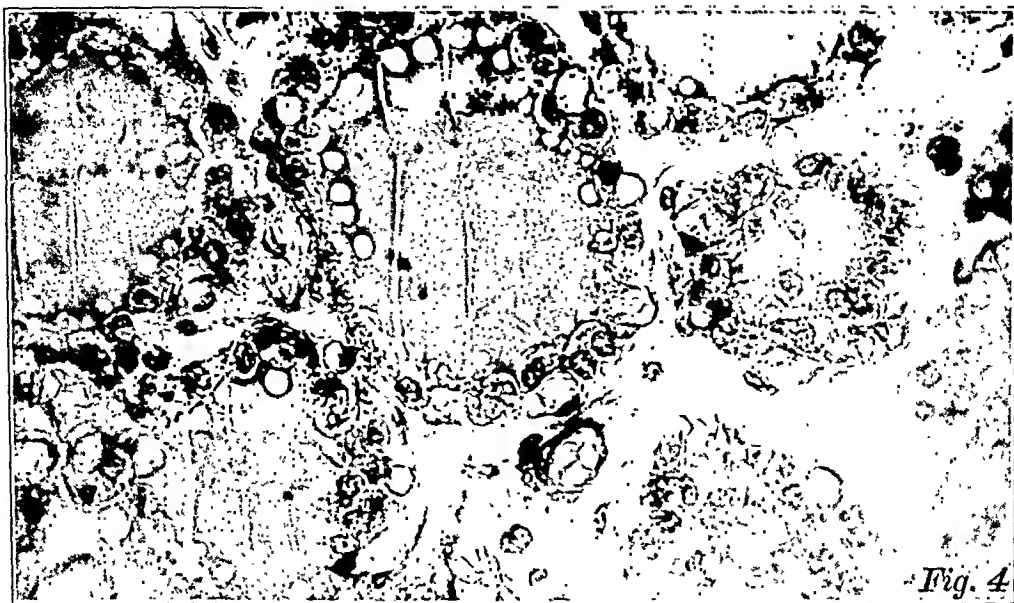


PLATE 64

- FIG. 4. Photomicrograph of a section of thyroid gland of a male guinea pig fed a chronic scurvy diet alone for 126 days. Irregular follicles with very high epithelium. Colloid extremely vacuolated and tending to disappear from the follicles and thus reduced in amount. Increased number of interfollicular cells but phagocytes are scarce. $\times 800$.
- FIG. 5. Photomicrograph of a section of thyroid gland from a female guinea pig fed a chronic scurvy diet 56 days before the administration of potassium iodide. Potassium iodide then given for 82 days. Union of colloid after the follicular wall is broken. Increase in rarely vacuolated colloid in the irregular follicles. Low, flat, thin epithelium about to break through in many places. Phagocytes are rare. $\times 800$.
- FIG. 6. Photomicrograph of a section of thyroid gland of a female guinea pig fed a chronic scurvy diet for 65 days before the administration of thyroid substance. Thyroid substance then given for 55 days. Increase in colloid and decrease in interfollicular cells, as compared to Figure 4. Colloid retracted but non-vacuolated. $\times 800$.



GLOMERULAR CHANGES IN ARTERIOSCLEROTIC CONTRACTION OF THE KIDNEY *

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In the course of systematic investigations on arteriosclerotic contraction of the kidney, I have paid special attention to the changes in the basement membrane of the glomerulus and its capsule.

The following communication is confined to degenerative changes and is not concerned with inflammatory processes. Furthermore, those degenerative changes that are due directly to lesions of the vas afferens are not considered. The method of glomerular destruction associated with hyaline or fatty degeneration of the vas afferens — whether due to obstruction of the blood stream or to immediate encroachment upon the glomerular capillaries — is so well known that my investigations could not possibly add anything new. It appears that less attention has been paid to degenerative changes not associated with visible disease of the vas afferens, in spite of their far greater frequency. The reason, apparently, is that the earlier stages of glomerular atrophy become conspicuous only with special stains. The Lee-Brown stain has proved quite adequate for this purpose and is simple in practice.

Two different forms of glomerular degeneration can be distinguished, a primary and a secondary. Both forms can lead to the same terminal destruction.

PRIMARY (SENILE) DEGENERATION

Axial Increase of Connective Tissue

The primary form consists of a thickening of the connective tissue framework of the glomerular tuft. When the basement membrane and the connective tissue are demonstrated by the Lee-Brown stain, the delicate subepithelial membrane stands out sharply delineated and is not thickened. The central axis of the glomerular lobule, on

* Received for publication December 18, 1934.

the other hand, is more marked, stains a deep blue and shows a blurred outline. Even under low power the lobulation of the glomerulus is accentuated by this broadening of the axial supporting tissue (Fig. 1). The fact that on cross-section the delicate basement membrane can be distinguished clearly from the thickened axis, on which it lies tangentially, proves definitely the separate existence of the latter as a mass of intercapillary connective tissue.

The presence of such a structure has long been denied, but the result of research in normal anatomy leaves no doubt that the glomerular tuft contains fibrillary nucleated connective tissue between the capillaries, in addition to the epithelial cells, the endothelial cells, and the basement membrane (Zimmermann, von Möllendorff and Borst).

In minor degrees of thickening this intercapillary connective tissue shows a fine fibrillary structure, but later becomes homogeneous and hyaline. The broadest masses of hyaline connective tissue are always found in the intraglomerular portion of the hilum. The other glomerular components (epithelium, endothelium and basement membrane) are quite normal. In the overwhelming majority of cases the vasa afferentia and precapillaries likewise are unaffected.

Since such a great number of glomeruli show the changes described above in otherwise normal kidneys, one might be inclined to regard this picture as a variation of the normal. There are, however, great variations of intensity and extent of this process in different kidneys. The degree of thickening and number of altered glomeruli both obviously increase with age. Although some arteriosclerotic and normal kidneys in old subjects show no thickening of the axial connective tissue, this feature was entirely absent in a control series of twenty individuals under 20 years of age. It proved impossible by counting those glomeruli to show any parallelism with any type of vascular change or with high blood pressure, which might possibly exist. Likewise, no relation can apparently be established to passive congestion of the kidney.

Although severe and extensive axial thickening was observed in 2 cases where the individuals were 32 and 45 years of age, nevertheless its greater frequency in older subjects is undoubted. This points to the conclusion that the change described cannot be regarded as a variation of the normal but is an expression of the aging process in the glomerulus.

Since MacCallum in a recent paper pays attention especially to the change of intercapillary connective tissue and states that the main group of such cases occurs in association with cardiac hypertrophy and arterial hypertension, I shall go more into detail in regard to this point.

It is true that the process of thickening of the wall of the vasa afferentia very often tends to enter into the glomerular tufts and that in these cases the secondary degeneration of the glomeruli is due to an increase in intercapillary connective tissue, which in later stages compresses the capillaries. However, it must be emphasized that the causal connection is confined purely to the arteriosclerosis. The relation between arterial hypertension and arteriolar sclerosis still is a question. The object of this paper is to stress the fact that the "axial thickening" is a far more common finding and may very well occur independently of changes in the arterioles. When comparing this change with the heart weight, I found among 46 cases in which I enumerated the glomeruli with thickened connective tissue: (a) 6 cases of severe thickening with a heart weight under 400 gm.; 3 cases of severe thickening with a heart weight over 430 gm.; (b) on the other hand, 7 cases with a heart weight of more than 500 gm. had a degree of axial thickening ranging from zero to about half as much as in the cases of the first group.

It can be definitely stated, then, that this condition is independent of arterial hypertension and cardiac hypertrophy.

MacCallum speaks in these cases of a growth of the mesangium, but my histological examination never revealed any increase of nuclei unless there was an inflammatory process associated with the axial thickening. Because of this I cannot convince myself of an actual axial growth.

Considering the details of the process, one can state that the degeneration in question begins at the hilum where the capillaries branch, and progresses towards the periphery (Fig. 2). While the thickening is frequently confined to the region of the hilum, I have never seen peripheral broadening of the connective tissue in a glomerulus with a normal hilum.

In consequence of this process, collapse of the capillaries occasionally develops. In some rare instances this collapse is confined to one glomerular lobe, of which the axis is definitely thickened, while its subepithelial membrane is still unaffected. One might, then, justifi-

ably assume that the collapse and the axial thickening are closely related. In the later stage of hyalinization, which of course involves the basement membrane, it is impossible to make any such distinction.

It should be mentioned that the thickening of the intercapillary connective tissue can occur also in connection with other processes.

We not infrequently encounter cases in which the intercapillary change is so marked and so extensive that one might be inclined to look upon this disease of the kidney as a characteristic lesion. A later paper will be devoted to a closer study of these kidneys. The axial thickening may develop in amyloid degeneration or subsequent to inflammatory lesions and can be demonstrated in the glomerular destruction which follows primary thickening of the basement membrane. Instances of the latter, however, as we shall see later, are decidedly uncommon. Axial thickening has been mentioned several times in recent papers (MacCallum, Schürmann and MacMahon), although associated with other conditions. It must be emphasized, however, that this lesion in its pure form is exceedingly frequent and independent of changes in tubules and blood vessels; hence it has been termed "primary degeneration."

SECONDARY DEGENERATION

Thickening of the Basement Membrane

This description applies to glomeruli whose basement membrane is thickened (for the most part uniformly) and has a wrinkled appearance. The glomeruli are simplified in structure as a result of atrophy and for this reason are easily recognizable by special stains under low power. These lesions of the glomerulus have been described repeatedly and in excellent detail by McGregor. I shall therefore take her conclusions as a basis for the following considerations.

McGregor introduced the term "hypertensive glomeruli," assuming that a close connection exists between this form of glomerular disease and arterial hypertension. Her investigations failed, however, to shed any light on the pathogenesis of the glomerular lesions. A definite relation to arteriolar sclerosis could not be established, as partial or complete hyalinization of the vessels could be found only at some distance from the glomeruli they supplied. The author

herself was apparently not satisfied with the morphological relationship as the basis of the glomerular lesion.

I have tried to confirm the results that McGregor obtained by counting the hypertensive glomeruli. It can certainly be shown that the number of these "membrane-thickened" glomeruli is considerably increased in kidneys with severe arteriosclerosis. One should, however, regard quantitative observations on histological preparations with more skepticism. In enumerating the glomeruli it is impossible to avoid subjective errors which are present in the "birds-eye view" method of estimation. We are easily led thereby to form an impression with a false sense of certainty. Even if sections are taken from several parts of the kidney the method is still inaccurate owing to topographical variations.

Thus, counting every glomerulus throughout the section, I found a high percentage of so-called hypertensive glomeruli in simple, scarred, atrophic, senile kidneys without any relation to high blood pressure. The difference between McGregor's and my results might possibly be explained by the varying size of the scars in which the hypertensive glomeruli are crowded. I encountered cases in which the so-called hypertensive glomeruli could not be demonstrated anywhere else but in the vicinity of an old scar (Figs. 3 and 4). According to McGregor's own statement, she believes that this glomerular change depends on a circulatory damage. As we shall see later, I agree entirely with this point. I also believe that the thickening of the basement membrane is due to an ischemic process.

I encountered several cases of severe hypertension with moderate arteriosclerosis of the kidney in which only very few glomeruli with thickened basement membrane were present. As is well known, arterio- and arteriolosclerosis are frequently found in association with a previous history of hypertension. This, in my opinion, however, can be interpreted as a coincidental relationship rather than one of cause and effect. Inasmuch as I have found the distribution of the so-called hypertensive glomeruli to be a focal one related to vessel change, and therefore probably caused by ischemia, I prefer the term ischemic glomerulus for this type of change.

Two types of glomeruli with thickened basement membrane can be distinguished. The first is characterized by additional thickening of the capsular membrane with only slight widening of the capsular space, because of atrophy of the glomerular tuft itself. The second

shows definite dilatation of capsular space with no thickening of the capsular membrane. The combination of both these types is possible but the differentiation between them is necessary since the common occurrence of the pure form of either type strongly indicates their different histogenesis.

*Ischemic Atrophy of the Glomerulus with Thickening
of the Capsule*

The majority of the vasa afferentia of this type of glomerulus are normal, although partial hyalinization of the vessel wall may be encountered occasionally, but not constantly, some distance proximally. In view of the inconstancy of this finding, it is most improbable that a direct relation exists between this type of glomerular lesion and degeneration of the arteriole or prearteriole. Furthermore, one can practically never recognize with certainty any associated thickening of the axial connective tissue.

It is obvious that the process from the beginning consists of the thickening of epithelial basement membrane, but the change is confined for the most part to the glomerulus and only exceptionally extends to the basement membrane of the tubules. This type of glomerular degeneration has often been noted on account of the associated thickening of the capsule, which is easily demonstrable by the common staining method hematoxylin-eosin and Van Gieson, (Tschistowitsch, Roth, Herxheimer, Fahr and Aschoff). The onset of the process is usually observed at the site of reflecture of the basement membrane of the capsule to the capillary tuft, but may sometimes be particularly marked at the pole opposite to the hilum (Fig. 5). The contributions in the literature are purely descriptive. The mode of development of this special form of glomerular atrophy is still in question.

On the negative side we can first state, contrary to the assumption of most authors, that in all these numerous cases capillary collapse is not the result of mechanical narrowing of the vas afferens. The vessel is intact and very frequently shows passive congestion with dilatation of the lumen (Fig. 6). This of course does not apply to the exceptions mentioned in the introduction.

Two modes of development are to be considered: (1) ascending, due to obstruction of excretion; and (2) primary circulatory damage of low degree.

We can exclude the first mode in the above glomerular degeneration since no dilatation of the capsular space or the corresponding tubules is present, and without this criteria such a conclusion would not be justified.

On the other hand, there is adequate evidence to support the second mode of development. Membrane-thickened glomeruli are aggregated in so-called incomplete infarcts (Fahr), that is, wedge-shaped areas where the glomeruli are crowded between atrophic tubules. Although in hematoxylin-eosin preparations the glomeruli frequently appear normal, the special stains show thickening of the basement membrane. At the apex of such wedge-shaped areas one can as a rule recognize the arteriosclerotic narrowed vessels.

The glomeruli with thickening of the basement membrane and capsule are almost invariably aggregated together, but even if they occur isolated the corresponding tubular apparatus is atrophic (Fig. 7). In a doubtful case, serial sections will reveal the association of the glomerular and tubular atrophy. The tubular basement membrane, however, is rarely thickened, and there is little more than a slight broadening of the loose interstitial connective tissue.

It is, therefore, desirable to discuss the relation of the tubular atrophy to the glomerular change. In the majority of the atrophic scars the obliteration of the glomerulus is considered to be primary, atrophy of the tubules occurring as a secondary process (Fahr, Aschoff, Loehlein, Stoerk, Jores and Herxheimer). Opinions differ, however, as to the mechanism. On the basis of Stoerk's assumption of a vascular unit, that is, of a tubular blood supply via the glomerulus, the tubular atrophy is of circulatory origin. Other authors (Jores, Herxheimer, and others) favor the hypothesis of a disuse atrophy. Aschoff's explanation of the tubular atrophy resulting from excretion of toxins by the glomerulus appears to be only a theoretical possibility.

Certain qualifications are made by Herxheimer and Fahr in special cases, since they pointed out the possibility of a combined atrophy depending on narrowing of the larger vessels, in which case tubular might precede glomerular atrophy.

It has only lately been emphasized by Staemmler that the above possibility is most frequent in arteriosclerotic contracted kidneys. The proof lies essentially in the fact that according to this author the glomeruli are fairly well preserved even when tubular atrophy is very

advanced. The special stains, however, reveal in such areas many glomeruli with a thickened basement membrane. The same holds for smaller areas which contain only a few nephrons.

It is not to be denied that a primary lesion of a vas afferens often enough leads to destruction of the glomerulus and subsequently to atrophy of the tubules. In view of the early changes in the glomerular basement membrane, one gets the impression that the significance of such processes in contraction of the kidney has been overrated. Hyalinization or fatty degeneration of the vas afferens, which in fact leads to narrowing of the lumen with subsequent damage of the glomerulus, in absence of sclerosis of the larger vessels, affords a rare exception. In this respect I disregard the frequent hyalinization of arterioles without change of their lumen. Thus, it is quite immaterial whether the tubules receive their blood supply via the glomeruli or directly (Elze and Dehoff). The essential point is that tubular atrophy is purely circulatory, as is evident from the sequence of events; atrophy of tubular epithelium and thickening of the capsule membrane can often be recognized before any change can be demonstrated in the glomerulus. Furthermore, the microscopic picture leaves no doubt that the process may encroach on the glomerulus from the capsule, especially at the hilum, without involving the vessel at all. It is striking how long the capillary epithelium is preserved.

The fact that the tubular epithelium is much more susceptible to nutritional disturbance than the glomerular capillary apparatus also makes this sequence of events most probable. It has recently been shown in a paper by Maatz that a relatively short constriction of the large renal vessels produces in the first place, and chiefly, tubular atrophy. I believe also that the same explanation applies to the "tubular kidney atrophy" which Baehr produced by injection of iodine, particularly as the vessels showed marked changes.

Finally, we must mention another possible mode of development of atrophic scars described by Fahr and later by Helpap; namely, ascending contraction due to primary sclerosis of the medulla. The histological resemblance of these cases to ascending pyelonephrotic contraction suggested this conception to Fahr. Definite proof, however, is difficult to obtain. Whether we assume with Fahr a collapse of the lower parts of the tubules, or whether we postulate pressure from without, we should expect to find stagnation of secretion with

subsequent ascending dilatation. This, however, usually does not occur.

On the other hand, we know that distention of the pelvis produces ischemia of the kidney. Hinman and Morison have given conclusive experimental illustrations of this. It appears, therefore, that the same mechanism which we postulate in incomplete infarctions might come into play in ascending infection or pyelonephrosis. The histological appearances are in fact extraordinarily similar. Histological differentiation is indeed possible only in the presence of a characteristic distribution of the inflammatory infiltration. The type of contraction is itself identical. Fahr's conception of ascending contraction following primary sclerosis of the medulla is only tenable if sclerosis of the large vessels can be excluded on the one hand, and urinary obstruction, on the other hand, is demonstrable.

Ascending Atrophy

This form of glomerular atrophy with thickening of the basement membrane not infrequently occurs in arteriosclerotic kidneys. Small and large cysts are present in the renal cortex, representing dilated capsular spaces, in which often only a residue of the glomerular tuft can be recognized. Although Beer in 1904 described in detail these small glomerular cysts and stated numerically that 31 per cent of all degenerating glomeruli underwent this cystic change, only scanty information about them is found in the text-books.

In my experience the process, though very frequent, seems to be less common than is claimed by Beer. This author did not take account of the fact that hyalinized glomeruli may disappear completely. This alone invalidates any method of comparative enumeration.

Staemmler and Masugi pointed out, and Moritz and Hayman furnished the experimental proof, that hyalinized glomeruli can disappear without leaving any trace. The basement membrane of the glomerulus in ascending atrophy is usually quite uniformly thickened and wrinkled, but in contrast to the findings in ischemic atrophy (Fig. 8) the capsule largely remains unaffected (Fig. 9). The vas afferens is almost invariably patent and the glomerulus shrinks more and more, although capillaries are wide open and filled with blood. Here, too, surprisingly enough, the epithelial cells are often well preserved to the end and not even flattened in every case. The excessive

dilatation of the capsular space which is filled with coagulum points to a primary obstruction to secretion.

In spite of careful examination, Beer could not find any actual occlusion at the outlet of the capsular space and therefore assumed that constricting bands of connective tissue might lead to obstruction some distance from the glomerulus. Indeed, if we follow these cysts in serial sections we observe that the dilatation need not be confined to the glomerular capsule but sometimes involves one or several loops of the convoluted tubules. Here, in fact, one often finds a band-like increase of connective tissue just where the dilated tubule ends, sometimes rather distant from the glomerulus, showing that passive stagnation of secretion might be verified histologically. I agree with Beer's statement that the picture is often so complicated that a single nephron cannot be traced, especially when, as is frequently the case, the glomerular cysts are grouped together. However, it is in this type of case that reticular, scarring fibrosis is seen to be the cause of stagnation (Fig. 10).

Aschoff's assumption that these cysts are due to a developmental abnormality is most improbable. They indeed resemble the dilatation of the capsular space, which is so frequently found, especially in the outer zone of the cortex of the kidney of young infants, and which undoubtedly is due to malformation. Aschoff believes that such cysts increase in size with age and become particularly visible when the kidney contracts in old age, or as a result of inflammation. This explains why they are relatively rare in kidneys of young individuals without arteriosclerosis and why they seem to be more common in old subjects. In such arteriosclerotic kidneys, however, we see the cysts in all stages of development and it is inconceivable that the capsular dilatation should be preserved from infancy to old age, especially as we know that many glomeruli become obliterated and disappear on account of this process in early childhood (Herxheimer).

Cystic degeneration of the glomeruli, which is beyond doubt caused by stagnation of excretion, frequently is due to scarring processes in the vicinity of the glomeruli. Furthermore, it is conceivable that in primary tubular atrophy the epithelial cells may obstruct the lumen. Cases in which the cysts are accompanied by a thickening of the capsule afford evidence of such a process actually taking place. For we have seen above that thickening of the capsule and atrophy of the tubular epithelial cells are associated together in ischemic

atrophy. This combined picture is frequently encountered in incomplete infarcts and scars (Fig. 11).

CONCLUSIONS

In arteriosclerotic kidneys the following degenerative changes can be recognized in the glomeruli:

1. Primary broadening and hyalinization of the intercapillary axial connective tissue. This very frequent change is interpreted as an aging phenomenon of the glomerulus and may lead to secondary damage to the glomerular capillaries.

2. Thickening of the basement membrane, which is always secondary, may be due to two different causes:

(A) Ischemic atrophy of the glomerulus which may result from:

(a) Direct encroachment of hyalinization of the vas afferens upon the glomerulus leading to collapse and degeneration of all glomerular elements.

(b) Narrowing of the larger vessels, producing slow circulatory atrophy of the tubules and glomeruli. This change, the most common in all forms of arteriosclerotic kidneys, is characterized by thickening of the capsule and basement membrane, frequently extending from the former to the latter. This thickening of the capsule is closely associated with atrophy of the tubular epithelium.

(B) Ascending atrophy. This is caused by obstruction of the corresponding tubules and is characterized by the thickening of the capillary basement membrane without thickening of the capsule and is associated with dilatation of the capsular space. This form usually is not observed in pyelogenic ascending contraction. The latter is interpreted mainly as an ischemic process, thereby explaining the fact that in this condition we so frequently encounter a high degree of capsular thickening (*vide* (A) (b)).

Tubular atrophy in arteriosclerotic kidneys is chiefly a circulatory one and essentially depends on changes in medium sized and larger vessels.

NOTE: The author wishes to express his gratitude to the Committee in Aid of Displaced Foreign Physicians and the Rockefeller Foundation for a grant which made possible the above investigation,

and is indebted to Dr. Frederic Parker, Jr., of the Mallory Institute of Pathology for research facilities and for his helpful advice and criticism.

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DESCRIPTION OF PLATES

PLATE 65

- FIG. 1. Accentuated connective tissue framework of the glomerulus "axial thickening."
- FIG. 2. "Axial thickening" progressing from hilum to periphery.
- FIG. 3. Old scar with hyalinized glomeruli and five so-called "hypertensive glomeruli."
- FIG. 4. Two "hypertensive glomeruli" of the same scar (Fig. 3) in high magnification.
- FIG. 5. Capsular thickening. Process encroaching upon basement membrane. Most of the basement membrane still delicate.
- FIG. 6. Same as Fig. 5. Vas afferens intact and congested.



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PLATE 66

- FIG. 7. Capsular thickening associated with tubular atrophy (membrane of tubules delicate). Basement membrane of glomerulus only slightly thickened.
- FIG. 8. Glomerulus with thickened basement membrane. Widening of the capsular space due to atrophy of the glomerular tuft.
- FIG. 9. Glomerular cyst without capsular thickening.
- FIG. 10. Group of glomerular cysts (without thickening of the capsule). Scar tissue towards the medulla.
- FIG. 11. Scar with combined ascending and ischemic glomerular atrophy.



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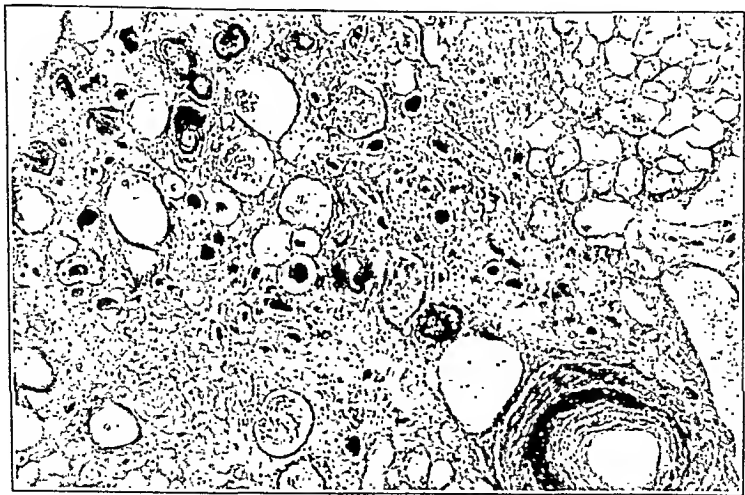
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REACTION OF PULMONARY TISSUE TO LIPIODOL *

R. DOUGLAS WRIGHT, M.S.

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The reaction of tissues to lipid substances is of increasing interest. The effect of vitamin D on tissue growth is well known. The local reaction of tissues to fats and fatty acids released from their protein envelopes and chemical unions respectively has been discussed by King,¹ Cohen,² Lee and Adair,³ and Stulz and Fontaine,⁴ and a simple phagocytosis by local connective tissue and wandering cells has been found. In the case of olive oil containing carotin injected into the peritoneum, Connor⁵ found a "foreign body" granuloma to be formed.

Sabin and co-workers^{6,7} have demonstrated that tuberculophosphate gives rise to an epithelioid reaction with Langhans' giant cells and marked caseation: in the environs lymphocytic and plasma cell collections occur. To phthioic acid a reaction more closely resembling the exudative type of tuberculous reaction was found.

Olive oil was found by Sabin⁶ to excite a "marked irritation" of the fibrous tissue. White⁸ found that when injected subcutaneously in the back of rabbits oleic acid gives rise to a cyst which becomes lined by squamous epithelium if it approaches skin appendages. When the injection is made into the mammary gland metaplasia of the epithelium to the squamous type always occurs.

The reaction of tissues to rapeseed oil containing a 35 per cent iodine is therefore of interest. Such reaction was found in the material here described. It was impossible to retain the lipiodol in the lungs of experimental animals so it could not be determined whether the iodine or the oil was the stimulus to the reaction found.

As no reference could be found in the literature to this type of reaction it is considered worthy of report.

Lipiodol injected into bronchi is usually expelled before any reactive changes to it are set up in the lung. In the lung of a patient into whose bronchi lipiodol had been instilled 12 months previously the oil was retained. At the time of introduction one of the main lateral

* Received for publication October 7, 1934.

branches of the right bronchus was seen to be blocked completely but the lipiodol passed readily into the lower branches. Roentgenograms taken a week before death showed the lipiodol still to be present in bronchiectatic cavities in the neighbourhood of a relatively radio-paque mass, especially in parts caudal thereto (Fig. 2). Figure 1 shows the manner in which this "trapping" of the lipiodol had taken place. Occlusion of the main bronchus had occurred by the growth of an epithelioma of a lateral branch of the right bronchus. The growth of this mass cranially probably explains the presence of lipiodol in the bronchi above the tumor.

The lungs were removed with the mediastinum. The right lung was adherent to the parietes over its whole surface but more firmly over the lower lobe. The diaphragm was separated from the lung with extreme difficulty when it was seen that this surface of the lung was covered with a thick white tissue. On section this was mainly distributed toward the mediastinum where it was almost an inch thick (2 cm.) but only 0.5 cm. thick at the lateral portions. The surface of this tissue was grey and shining; no fluid exuded from it. The texture was homogeneous, the consistence was harder than mucoid tissue and softer than cartilage. It was very firm and elastic.

The cut surface presented a large, rounded epidermoid carcinoma of a lateral branch of the main bronchus growing in a massive manner into the lung and main bronchus. No metastases were present in the mediastinal glands. The lung parenchyma about this tumor was compressed and fibrous. In the lower portion of the lobe it was not compressed but presented no evidence of alveolar structure on macroscopic observation. Throughout there were lobulated areas of fawn-colored material which stood out in marked contrast to the deep reddish brown of the parenchyma. These masses varied in size from a few mm. to 1 cm. in cross-section. The edges of these areas were definite but slightly blurred. The central zone was essentially homogeneous. The most noticeable feature of these areas was the regularity of distribution and of lobulation in them. The main bronchi were dilated, the mucosa rough and reddened. Blood vessels stood open in the neighbourhood of the tumour.

Frozen sections were taken to include the diaphragmatic surface and the lung with several of the buff-colored areas adjoining it. These were stained with Sudan III which stained the lipiodol deep red. The sections were then stained with haematoxylin.

No fat occurs normally in these areas so that all the material stained by the Sudan III can be interpreted as abnormal. This material can be freed with a needle from the section; it is then seen to be in the form of liquid oil droplets. The areas are, to judge from the roentgenogram taken before death, radiopaque. On this evidence then I will assume that the substance stained by the Sudan III in these sections is lipiodol.

These areas are represented in Figure 4 and Figure 5. The red oil droplets are represented deep black, a green filter and orthochromatic plates being used. In Figure 5 the edges of the two areas of lipiodol accumulation are seen. The lipiodol is in more or less confluent droplets, forming a dense reticulated mass. In Figure 4 these masses are seen to run over many cell areas and are not actually enclosed by any one cell. At A, however, very fine droplets are seen to be included in a macrophage.

When the lipiodol is removed by a fat solvent and the section counterstained by eosin the areas where the lipiodol occurred are seen to be made up of a lace-work of finely reticulated cells with small round nuclei (Fig. 3). The cytoplasm is extremely scanty, slightly vacuolated and extending in fine interconnecting strands from one cell to the other. No collagen fibrils (Van Gieson) were demonstrated arising from these cells.

The lipiodol appears to be enclosed in a foam of cellular syncytium. The origin of these cells is probably the supporting tissue of the bronchi.

Between these areas the remnants of lung parenchyma are seen as small channels with thick fibrous walls.

No bronchi or epithelium are found. The whole of the lobe is remarkably avascular. Even in a section vessels are rarely seen.

The thick hyaline tissue on the diaphragmatic pleural surface is a peculiar form of granulation tissue (Fig. 6). Small, well developed capillaries are seen running at right angles to the lung surface. They are widely separated by a material which takes the eosin poorly, but Van Gieson's stain shows a fine collagenous reticulum. It does not take stains for mucus (thionine and Mayer's mucicarmine) at all. There was no selective staining by iodine or gentian violet. Scattered through it are macrophages in small numbers. Some of these contain small droplets of lipiodol. Most of this substance is, however, extracellular and is almost entirely close to the capillaries in fine

droplets. To liberate them from this situation it is necessary to tear the tissue with needles; they could not have been deposited here during the preparation of the section. Such tissue is unique as far as can be determined from the literature. That it is a reaction to the lipiodol appears probable.

The two outstanding features of this specimen are the reaction of the reticular tissue of the lung to the prolonged presence of lipiodol and the reaction of the pleural tissue.

The cavities which were demonstrated in the first roentgenogram were still diagnosed as such 12 months later. In the meantime they had, however, become completely obliterated and sterile, as judged by staining. This result is certainly due to a tissue reaction to lipiodol which has been retained a long time. The relationship to lipiodol of the peculiar pleural reaction is one of probability only. However, the occurrence of lipiodol in the tissue and the most unusual type of this tissue make the probability great.

SUMMARY

The reaction to lipiodol retained for long periods in the bronchi is the development of lipophages from the supporting connective tissues. No epithelial reaction occurs.

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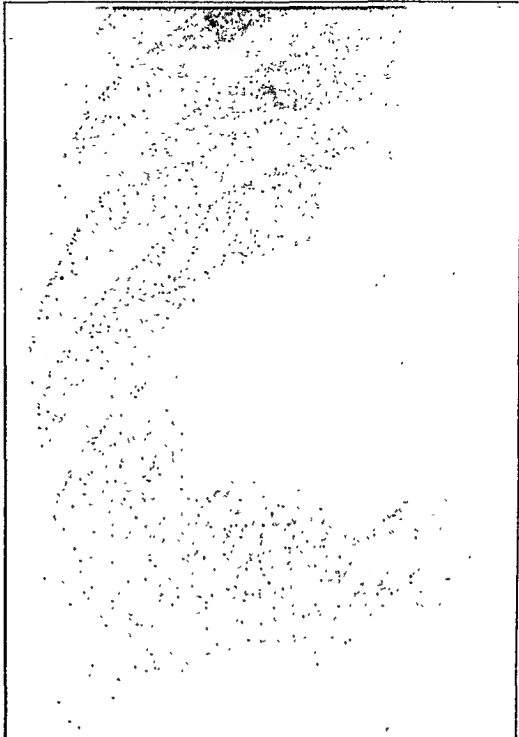
DESCRIPTION OF PLATE

PLATE 67

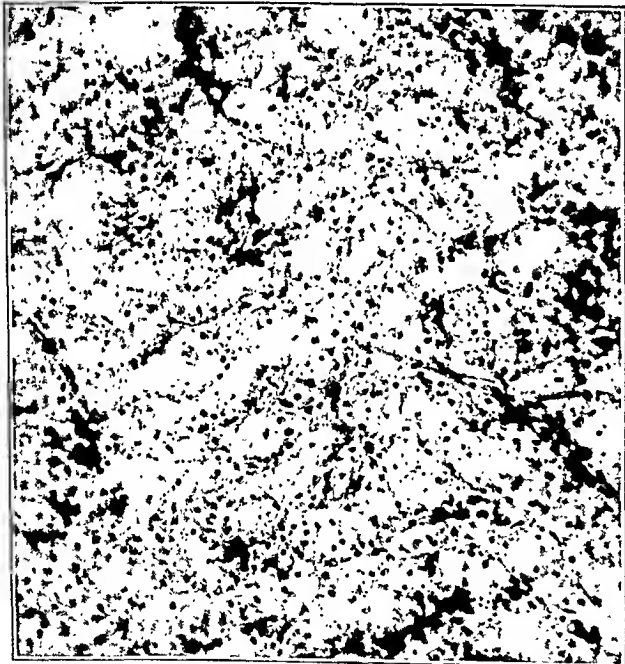
- FIG. 1. Coronal section through right lower lobe. An epithelioma is growing into the right main bronchus which is dilated. Lobulated masses of lipiodol and the pleural reaction may be seen on the cut surface.
- FIG. 2. Roentgenogram of chest 1 week before death, showing opaque circular mass of tumor and presence of lipiodol still present in bronchi.
- FIG. 3. The reticulated cells left after dissolving lipiodol from the lung. The openess of the cytoplasmic mesh is well shown. Haematoxylin and eosin stain. $\times 160$.
- FIG. 4. Lipiodol in relation to cells is shown. For the greater part it extends in masses over several cell areas but at A it is present as droplets in a macrophage. Sudan III and haematoxylin stain. $\times 400$.
- FIG. 5. The edges of two lipiodol masses. Sudan III and haematoxylin stain. $\times 40$.
- FIG. 6. Section of pleural reaction tissue. Stained with haematoxylin and Van Gieson. The reticulation of collagen fibres is shown, although these do not stain with eosin. Macrophages and capillaries are also evident. $\times 160$.



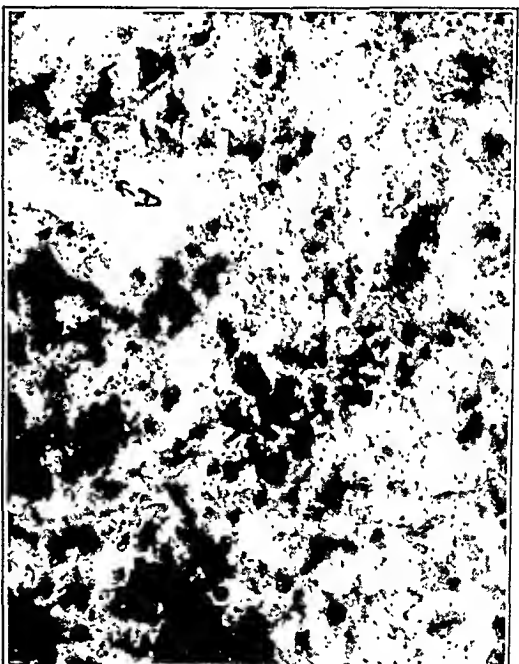
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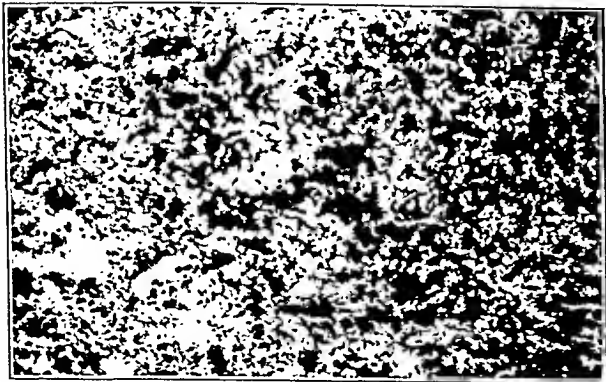
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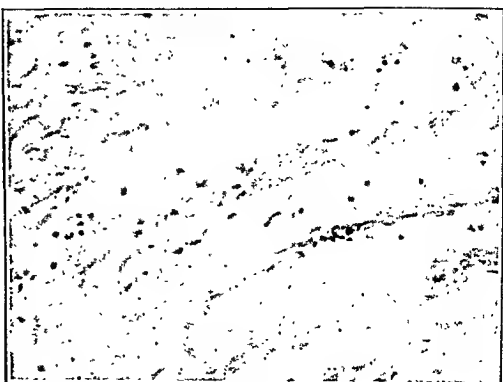
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Wright

Reaction of Pulmonary Tissue to Lipiodol

INFARCTION OF THE LIVER *

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True infarcts of the liver are so uncommon and the mechanism of their production is, for the most part, so poorly understood that a complete description of 2 additional cases of infarction of the liver seems warranted. Winternitz in 3500 autopsies at Johns Hopkins Hospital did not encounter a single case, Chiari saw 2 cases in 21 years, and many pathologists of wide experience have never seen a case. In a series of about 23,000 autopsies at the University of Minnesota there are but 2 cases.

The infrequency of infarction of the liver intrigued early pathologists. Von Recklinghausen suggested that the double blood supply of the liver was in some way responsible. Following him a number of authors such as Rattone and Mondino, Osler, and Leusden, on the basis of both pathological and anatomical findings and experiments, declared that simultaneous occlusion of both the hepatic artery and the portal vein was necessary to produce true infarction of the liver. This view gained such a firm foothold that it has not been shaken, despite the evidence to the contrary which has accumulated in the past 40 or more years.

The first insight into the true mechanism of infarction of the liver came as a result of the work of the early experimental physiologists. Simon de Metz in 1828 ligated the hepatic arteries in pigeons. He noted that there was no disturbance of the secretion of bile after cutting off the arterial supply of the liver, but he said nothing of necrosis of the liver. In 1857 Kottmeier ligated the hepatic arteries of frogs and rabbits. In frogs there resulted fatty degeneration of the liver; in rabbits he found light yellow, soft areas scattered through the parenchyma of the liver. However, he maintained that these areas of softening were not necessarily produced by the ligation of the liver arteries since he found similar areas in the liver of an animal with a normal arterial circulation.

* Received for publication October 11, 1934.

TABLE I

Cases of Ligation of the Hepatic Artery (or of its Branches) with Infarction of the Liver

Author	Date	Sex	Age	Vessel	Cause of ligation	Pathological lesions in liver
Kehr, H.	1903	M	37½	Main hepatic artery	For aneurysm of right hepatic branch	Necrosis of right border of liver
Bercsnegowski, N.	1906	F	46	Right hepatic artery	Bleeding during resection of carcinoma of gall-bladder	Total necrosis of right half of liver
Guibé, and Herrenschildt, A.	1907	F	22	Left hepatic artery; left branch portal vein	Resection of hydatid cyst left lobe liver	Total necrosis of left lobe
Narath, A.	1909	F	46	Left hepatic artery	Resection of chronic gastric ulcer	Widespread necrosis of left lobe
Kehr, H.	1909	F	36	Hepatic artery proper	During cholecystectomy and removal of common bile duct stone	Necrotic tissue removed on eighth day
Versé, M.	1909	F	48	Main hepatic artery	Resection of carcinoma of stomach	Widespread early ischemic infarction
Wendel, W.	1911			Hepatic artery beyond right gastric	Resection of carcinoma of stomach	Almost total necrosis of liver and gall-bladder
Wilms, M.	1912	M	47	Branch of hepatic artery (ligature?)	Resection of carcinoma of stomach	Extensive necrosis of left lobe
Schütz, H.	1914	M	48	2 branches of hepatic artery	Resection of carcinoma of stomach	Almost total necrosis of left lobe
Kretz, R.	1916			Right hepatic artery	During an operation	Necrosis of two-thirds of liver

Holst, S. F.	1920	M	66	Main hepatic artery	Resection of carcinoma of stomach	Left lobe small, soft, with areas of necrosis
Ritter, A.	1922	M		Right hepatic artery	Resection of carcinoma of stomach	Necrosis of right lobe of liver
Newcomb, W. D.	1930			Hepatic artery	Operation	Central necrosis of each liver lobule
Gerlach, W.	1930	F	30	Right hepatic artery	For severe bleeding after cholecystectomy	Numerous areas of necrosis
Graham, R. R., and Cannell, D.	1933	M	49	Main hepatic artery	Resection of carcinoma of stomach	Multiple necrotic areas in left lobe
Shann, H., and Fradkin, W. Z.	1933	F	48	Hepatic branches (?)	During cholecystectomy	Grayish-white mass 10 x 5 cm. sequestered
Kerr, R. W.	1933	F	57	Right hepatic artery. Portal vein	During cholecystectomy	Massive infarction of right lobe

Postoperative Infarction of Liver not due to Ligation of Hepatic Artery

Kausch	1904	F		A large branch of hepatic artery	Thrombosis during cholecystectomy	Slight necrosis of liver
Cioni, C.	1932	M	49	Left hepatic artery	Thrombosis following gastroenterostomy	Anemic infarction of left lobe

Betz (1863) in his experiments on dogs came to the conclusion that ligation of the common hepatic artery at its origin from the celiac was not dangerous. If, however, the artery was tied beyond the origin of the pancreaticoduodenal branch, fatty degeneration of the liver was present after 48 hours. In 1876 Cohnheim and Litten ligated all the arterial vessels present in the hepatoduodenal ligaments of rabbits. There resulted a total necrosis of the liver. The tying of only one branch of the hepatic artery resulted in necrosis of the corresponding lobe alone. Following this, Litten (1890), Pick (1890), De Dominicis (1891), Hahn and co-workers (1893), Janson (1895), Doyon and Dufourt (1898), Dujarier and Castaigne (1899), Ehrhardt (1902), Tischner (1904) and others investigated this problem in various animals with widely varying results and even more diverse interpretations.

It remained for von Haberer (1906) to clarify the subject. This investigator ligated the hepatic arteries of dogs, cats and rabbits. He injected a colored mass into the blood vessels after sacrificing the experimental animals in order to trace the vessels to the liver more readily. In this way he was able to establish that in those animals that did not die in a short time, the liver received its arterial blood from some source other than the hepatic artery. He performed several types of ligation: of the common hepatic artery as close to the celiac axis as possible, of the main hepatic artery distal to the origin of the right gastric, and of the right gastric and the gastroduodenal with resection of the arterial system between the ligatures. In a few cases the animals survived even the last procedure, and in these it was possible to demonstrate markedly developed diaphragmatic arteries. If the arterial supply was actually cut off, the animal promptly died of necrosis of the liver.

Many workers subsequently reinvestigated this problem in a large variety of animals (Bainbridge and Leathes, 1907; Nicolleti, 1910; Whipple and Sperry, 1909; Steckelmacher, 1913; Behrend, Radasch and Kershner, 1922; Loeffler, 1927; and others).

Of the recent studies, that of Cameron and Mayes (1930) deserves special mention. These investigators ligated the hepatic arteries of rabbits at various sites. They were able to corroborate the finding of necrosis of the liver following ligation of the main hepatic trunk. In addition they studied microscopically, as well as in gross, the stages in the development of the necrosis in the livers of their animals.

They were able to demonstrate definite necrosis in about 15 hours. The necrosis was usually massive and affected all zones of the liver lobules uniformly. A striking feature of their study was the relative immunity of the bile ducts and of the other structures within the portal canals from the necrotic process. Thrombi, particularly within the venous branches, were frequent, but by no means a constant finding.

There is evidence similar in nature to that described in experimental animals for the pathogenesis of infarction of the liver in man. Accidental ligation of the hepatic artery or of one of its branches, and purposeful ligation of the artery, chiefly for aneurysms, have provided us with a good insight into the importance of the hepatic arterial supply for the liver of man, as well as with most of the recorded cases of infarction of the liver.

I have been able to collect only 42 such cases from the literature. That they are more common is indicated in the opinion expressed by Behrend (1920) that "some unexplainable deaths following the operation of cholecystectomy may be due to ligation of the hepatic artery."

Kehr in 1903 ligated the main hepatic artery for an aneurysm of its right branch. The right border of the liver became necrotic and a strip 2 cm. wide ultimately separated. The patient recovered. Beresnegowski (1906) reported a case in which Tichow ligated the right branch of the hepatic artery during the course of an operation for carcinoma of the gall-bladder. There followed a total necrosis of the right half of the liver with death of the patient in 72 hours. Guibé and Herrenschmidt (1907), Narath (1909), Wendel (1911), Wilms (1912), Kretz (1916), Holst (1920), Ritter (1922), Gerlach (1930); Newcomb (1930), Graham and Cannell (1933) and Kerr (1933) report cases of ligation of the main hepatic artery or of one or more of its primary branches during the course of operation, with more or less complete infarction of the liver. Descriptions vary from those of multiple, anemic, sharply circumscribed areas of necrosis to total necrosis of the liver. This seems to prove that interruption of the arterial supply to the liver produces infarction. But how are we to explain the cases of ligation of the hepatic artery in man with recovery? Palacio (1898), Kehr (1903, 1909, 1913), Bakes (1904), Alessandri (1908), von Haberer (1909), Wendel (1911), Friedman (1912), Bertram (1913), Anderson (1919), Käding (1919), Ritter (1922),

Smith (1921), von Hofmeister (1922), and Shann and Fradkin (1933) all have reported cases of ligation of the hepatic artery or of its main branches with recovery of the patient.

On critical examination these cases fall into three groups. The first includes the cases of Palacio, Wendel and von Haberer, who ligated one branch of the hepatic artery preparatory to removal of the corresponding lobe of the liver. Obviously no necrosis could be expected in these cases. The second group comprises cases in which the hepatic artery or one of its branches was ligated during the course of some operative procedure in the upper abdomen (cholecystectomy, gastric resection, and so on). Of these the main hepatic arteries were ligated in only 2 cases (Ritter, 1922, and von Hofmeister, 1922). In the others a branch or branches of the hepatic artery were ligated. The possibility of anomalous branches could not be ruled out in these cases since the patients recovered. The third group includes the cases in which the hepatic artery or its branches were ligated in the removal of an aneurysm. Of these again only two involved ligation of the main hepatic artery (Kehr, 1903, and Käding, 1919). In this group there exists, too, the possibility of anomalous hepatic vessels. Moreover, particularly in the aneurysmal group, because of the relatively slow obstruction of the hepatic vessels, it is probable, as Segall has shown, that a collateral circulation is established especially with the diaphragmatic arteries, but also in part with the artery of the ligamentum teres, the right gastric or gastroduodenal arteries, or with arteries in adhesions between the abdominal viscera and the liver capsule. Cameron and Mayes (1930) have shown that in the rabbit methylene blue injected into the right internal jugular vein stains the liver even after the structures in the hepatoduodenal ligament and the hepatic veins are tied off. The collateral circulation responsible for the staining they conclude "is an important factor in maintaining the life of considerable parts of the liver after complete obstruction of the hepatic artery."

Narath and Ritter, on the basis of their experience with ligation of the hepatic artery or of its branches in humans, and Segall upon the basis of his studies of injected specimens of human livers, agree that the rules of surgical ligation established by von Haberer on experimental animals are valid for man.

Narath stated these rules as follows:

(1) The ligation of the main hepatic trunk is permitted providing at least one collateral is uninjured.

(2) The ligation of the hepatic artery proper before the origin of the right gastric artery is permitted if necessary. It may produce small areas of liver necrosis.

(3) The ligation of the hepatic artery proper is not permitted because of the great danger of liver necrosis. Exception — peripheral aneurysms.

(4) The ligature of a branch of the hepatic artery is not permitted, especially in patients with weak hearts.

We may conclude from these observations that infarction of the liver is produced in man by cutting off the arterial supply, just as has been amply demonstrated in experimental animals.

Let us examine now the group of cases in which infarction of the liver has occurred spontaneously, leastwise in the absence of any operative interference. Omitted from consideration here is the group that has been called "pseudoinfarcts." This includes cases of necrosis of the liver following injury. In these, as Zimmerman (1930) points out, there always exists the possibility that the necrosis of the liver cells is the direct result of the trauma. The so-called "atrophic red infarcts" which occasionally follow thrombosis of the portal vein have also been omitted because these have been shown to be due to atrophy and not to true necrosis. Finally, all cases of necrosis caused by thrombosis of the portal radicles have been omitted; first, because the mechanism of their production is quite different from that of the group under consideration; and second because, as Chiari pointed out long ago, the microscopic picture resulting from such thrombosis is quite different from that following interruption of the hepatic arterial supply.

I have been able to collect from the literature 52 cases that fulfill the essential requirements of anemic infarction of the liver. In these 52 cases the etiological factors may be divided as follows:

(1) Embolism of hepatic artery.

(a) subacute bacterial endocarditis	13
(b) mural thrombus	4
(c) paradoxical embolism	2
(d) thrombus in an aneurysm	2
	—
Total embolic	21

(2) Periarteritis nodosa of branches of hepatic artery	22
(3) Thrombosis of hepatic artery (three in aneurysms)	6
(4) Endarteritis of hepatic artery	2
(5) Hypoplasia of hepatic artery	1

TABLE II

Cases of Infarction of Liver (Not Including Periarthritis Nodosa)

Author	Date	Sex	Age	Mechanism of infarction	Pathological lesions in liver
Ross, G., and Osler, W.	1877	M	yrs. 21	Thrombus in aneurysm at bifurcation of hepatic artery	Numerous areas of necrosis with subsequent abscess formation
Obermüller, J.	1886	F	60	Thrombosis of multiple aneurysms of small branches of the hepatic artery	Multiple anemic infarcts
Orth, J.	1887			"Endocarditis diptheroides" with emboli to the liver	Faint yellowish, round infarct surrounded by a bright red zone
Rattone, G.	1888	F	young	Endocarditis secondary to puerperal sepsis with emboli to the liver	A small reddish brown infarct
Rattone, G.	1888	M	66	Obliterative endarteritis and thrombosis of hepatic branches; thrombosis of branches, portal vein	Two small "hemorrhagic" infarcts
Köhler, B.	1891	M	20	Ulcerative endocarditis; embolus in branch of hepatic artery	Multiple abscesses and a zone of necrosis
Ogle, C.	1895			Embolus from aortic valve blocking hepatic artery at its bifurcation	Multiple buff-colored infarcts from size of pea to hazelnut
Chiari, H.	1898	F	52	Mitral endocarditis. Embolus in hepatic artery	Complete necrosis of liver
Chiari, H.	1898	F	27	beyond right gastric. Emboli in smaller branches of hepatic artery	Multiple infarcts, some as large as a hazelnut
Castaigne, J.	1899			Cardiac thrombus; multiple emboli to liver	"Hemorrhagic" infarction of liver
Brion, A.	1901	M	15	Four intrahepatic aneurysms	Necrotic areas in the liver
Baldwin, F. A.	1902	M	39	Mural thrombus in left auricle	Multiple anemic infarcts
Ruczyński, B.	1905	F	40	Mural thrombus of right ventricle; patent foramen ovale; emboli in hepatic artery	Multiple zones of liver necrosis
Sotti, G.	1906	F	67	Aortic endocarditis. Thrombosis of portal vein and hepatic artery	"Hemorrhagic" infarction of left lobe and part of right

Reichmann	1908	M	26	Multiple emboli from a thrombosed aneurysm on main hepatic artery	Multiple infarcts of liver; many show cavity formation
Versé, M.	1909	F	26	Thrombosis central and hepatic veins with extension into hepatic artery and portal vein	Anemic infarcts in areas not involved by "more extensive process"
Herrmann, A.	1910		76	Thrombosis of branch of hepatic artery	A yellowish white dry area in right lobe
Dean, G., and Falconer, A. W.	1912	M	22	Aneurysm of hepatic artery, chiefly right branch	Two-thirds of right lobe necrotic; few cyst-like cavities in necrotic areas
Schütz, H.	1914	F	22	Aortic endocarditis; emboli to liver	Yellowish brown area of necrosis size of a hazelnut
Kretz, R.	1916	M	30	Acute endocarditis	Six anemic infarcts of liver
Wiessner, J. M. P.	1917	M	56	Mural thrombus in aorta with embolism to medium sized branches of hepatic artery	Multiple yellowish areas of necrosis, some as large as a walnut
Askanazy, M.	1918	F	71	Mural thrombus of aorta (?) with embolism of anomalous hepatic artery	Multiple anemic infarcts of liver
Mittasch, G.	1924	M	61	Emboli from auricular thrombi and possibly from mitral valve	Multiple "hemorrhagic" infarcts
Orlandi, N.	1924	F	26	Chronic mitral disease; mural thrombi in left auricle and ventricle with emboli to liver	Numerous grayish infarcts in right lobe
Orlandi, N.	1924	M	36	Embolism of hepatic arteries from bacterial vegetations on aortic valve	Multiple necrotic areas in both lobes
Orlandi, N.	1924	F	16	Thromboendarteritis with mycotic aneurysms of hepatic arterial branches	Multiple anemic infarcts
Hampeln, P.	1924	M	55	Infectious thrombosis of hepatic artery and its branches	Multiple infarcts of liver
Gerlach, W.	1930			Paradoxical embolism following thrombosis of femoral and iliac veins, and lower vena cava	Infarction of lower part of right lobe
Zimmerman, H. M.	1930	F	31 hrs.	Hypoplasia of hepatic artery; thrombosis of portal vein	Infarction of left lobe
Cioni, C.	1932	F	49	Recent ulcerative endocarditis of mitral and tricuspid valves; mural thrombosis of left atrium. Embolism of right hepatic	Infarction of right lobe
Pass	1934	M	24	Compression of main hepatic artery by extension from carcinoma of stomach	Multiple areas of infarction

The most common single cause of infarction of the liver, at least in the cases reported, is evidently periarteritis nodosa of the branches of the hepatic artery. In 170 case reports available, of the approximately 200 cases of periarteritis nodosa reported in the literature, there were found 22 cases of infarction of the liver.

In a few cases there was a concomitant endophlebitis or thrombosis of the portal vein or of its radicles. These cases would tend to support the contention of Rattone and Mondino, Leusden and Osler that occlusion of both the hepatic artery and the portal vein was required to produce infarction of the liver, were there not many more cases reported in which the arterial supply of the liver alone was disturbed.

Some authors, notably Wiessner (1917), have assumed that infarction of the liver following occlusion of the hepatic artery occurs only after necrosis and thrombosis of the portal venules with cutting off of the portal venous flow as well as of the hepatic arterial supply. Loeffler (1927) has recently reopened the whole controversy as to the relative importance and function of the hepatic arterial and of the portal venous supplies to the liver by stating that the hepatic arterial blood supplies the bile ducts and the walls of the portal venous radicles alone and that necrosis of the liver cells following occlusion of the hepatic artery is, therefore, really the sequel to necrosis of the bile ducts and of the portal vein.

However, it has been estimated that 35 per cent of the blood of the liver is supplied by the hepatic arteries, whereas the structures of Glisson's capsule constitute at most 10 per cent of the liver substance (Pfuhl, 1932). Furthermore, whereas the liver constitutes only 3 per cent of the body weight, it receives 5.1 per cent of the aortic blood through the hepatic arteries alone. Moreover, experiments with the Eck fistula have shown that the hepatic artery alone can supply the entire demand of the liver. These facts plus the observations of Cameron and Mayes of the relative immunity of the portal canals in necrosis of the liver following experimental ligation of the hepatic artery in animals, render it probable that the hepatic arterial blood takes part directly in the supply of the liver lobules.

That the portal vein plays a relatively minor rôle in the supply of the liver has been appreciated by some for many years. Many workers have reported accidental finding of completely thrombosed portal veins at autopsy, and the experimental work, although not altogether

unequivocal, nevertheless indicates that ligation of the portal vein in experimental animals is usually uneventful. Even simple atrophy of the liver or so-called "atrophic red infarcts" follow obstruction of the portal vein only when there is a concomitant impairment of the arterial circulation, as in a general heart failure. Hence, one would not expect the portal venous supply to be a very important factor either in the causation or in the prevention of necrosis of the liver.

TABLE III

Cases of Infarction of Liver with Periarteritis Nodosa of Hepatic Branches

Author	Date	Sex	Age	Pathological lesions in liver
Mönckeberg, J. G.	1905	M	18 yrs.	Several brownish, irregular infarcts
Versé, M.	1907	M	33	Multiple infarcts
Longcope, W. T.	1908	M	35	Multiple infarcts
Versé, M.	1909	F	19	Multiple anemic infarcts
Cameron, H. C., and Laidlow, P. P.	1918	M	27	A white infarct 4 x 3 cm. in left lobe
Kroetz, C.	1921	M	39	Multiple infarcts
Marinesco, M. G.	1923	M	28	Multiple small areas of necrosis
Pol	1925	F	23	Anemic infarcts
Baló, J.	1926	M	23	Recent and old infarcts
Christeller, E.	1926	F	29	Numerous anemic infarcts
Christeller, E.	1926	M	31	Anemic infarcts, chiefly subcapsular
Harbitz, F.	1927	M	32	Irregularly distributed areas of necrosis
Weigeldt, W.	1927	M	39	Anemic infarcts
Blum, K.	1929	M	43	Multiple infarcts
Arkin, A.	1930	M	55	Dark red depressed areas consisting of necrotic liver parenchyma
Arkin, A.	1930	M	50	Numerous, irregular, dark red infarcts
Arkin, A.	1930	M	34	Numerous gray-red infarcts
Arkin, A.	1930	M		Liver looks like syphilitic hepar lobatum (healed infarcts?)
Vance, B. M., and Graham, J. E.	1931	M	21	Multiple wedge-shaped anemic infarcts
Jäger, E.	1933	M	39	Anemic infarcts
Pass	1935	M	27	Multiple infarcts

Although we have demonstrated that the process of infarction is the same in the liver as in the spleen or kidneys, we have not shown why it is so rare in the liver. There are four observations that may help account for its rarity. The first is that the arrangement of the hepatic arterial supply is such that it renders embolism of the liver unusually difficult. The hepatic artery describes an arc of about 180° in its course to the liver. Askanazy was so impressed with the protective property of the peculiar vascular course that, finding a

case of embolism of the hepatic artery with infarction of the liver, he carefully dissected out the artery and was able to prove that it arose in his case from the aorta and passed directly to the hilum of the liver. Perhaps similar anomalies might have been found in some of the other cases of embolism of the hepatic arteries had careful examination of these vessels been made.

The second fact is that, as we have pointed out above, a collateral circulation protects the liver.

A third is the observation of Chandler (1920), who noted that the liver cells of dogs withstood surprisingly well the temporary anemia (3-12 hours) following ligation of both the hepatic artery and the portal vein. Even after 12 hours there was no necrosis of liver cells, but there was a marked fatty degeneration of the central half of the liver lobules. He concluded that "this power of the hepatic cells to resist local anemia probably accounts for the infrequency of infarcts in the liver." We must not examine this experiment too critically, however, for we are likely to discount its significance on the basis of a probable collateral arterial supply.

The final fact is that, as Cameron and Mayes have shown experimentally, infarcts of the liver frequently become converted into abscesses. The pathologist confronted at autopsy with multiple abscesses of the liver cannot know that these have earlier been infarcts.

There are a few cases reported which may conceivably represent healed infarcts of the liver. These include such cases as that of Ledieu who found an aneurysm the size of a hazelnut on the main trunk of the hepatic artery. The aneurysmal cavity was occluded by a firm clot. The liver showed cirrhosis. The increased fibrous tissue in this case might represent scars of healed infarcts (see Cameron and Mayes). Arkin reports a case of healed periarteritis nodosa with a small liver resembling a syphilitic *hepar lobatum* evidently produced by healing of infarcts. Cases of this kind are, however, for the most part so difficult to interpret, particularly in the absence of careful microscopic reports, that I shall dismiss this group without further mention.

Finally I wish to add 2 cases of multiple anemic infarcts of the liver.

CASE REPORTS

CASE 1. The patient, a white male aged 27 years, was admitted to the hospital on March 8, 1924, complaining of moderate pain in the abdomen, shifting in character, pain in the back and legs, and weakness. One month previously while helping to lift a heavy log, he had slipped and had had to use all of his strength to keep from falling. At that time he experienced a sudden severe pain in the abdomen, which confined him to bed. He had found it necessary to stay in bed since, because of pain and some vomiting.

He had never been ill before except for an infection of the left hand in November, 1923. There was no history of any venereal disease. For the two months just preceding his illness he had been working in a logging camp.

On physical examination his tongue was moderately coated, his teeth poor. The heart and lungs were normal. There was some tenderness in the epigastrium and in the left lumbar region on deep pressure. The entire abdomen was tympanitic with the exception of the left lumbar region which was dull. There seemed to be a slightly increased resistance to pressure in the left lumbar region, but the abdomen was otherwise soft.

Laboratory examination on March 9 showed: urine — amber color, acid, specific gravity 1010, negative for albumin and sugar. The leukocyte count on March 11 was 30,000.

The patient vomited several times after admission to the hospital. The abdominal pain shifted from one side of the abdomen to the other and was so severe that it required morphine for relief.

On March 15, 1924, a laparotomy was performed. Only a small amount of dark bloody fluid was obtained, but no lesion could be demonstrated. Drains were inserted and the wound was closed.

The patient expired on March 26, 1924. His temperature on admission was 101° F. It varied between 97 and 101 during his hospital stay. Clinically it was felt that the patient had had an intestinal hemorrhage.

AUTOPSY REPORT

The body was that of a poorly nourished, adult male. A rather recent, almost completely healed surgical incision extending from the umbilicus to the symphysis pubis was present. Rigor was present. There was no edema. A distinct bluish discoloration was seen over the chest and abdomen.

The omentum was brownish green and was attached by soft adhesions to the hepatic flexure of the colon. A small white nodule just lateral to the hepatic flexure was observed. The posterior abdominal wall on the left was pushed forward by a large hematoma. There were a number of soft adhesions between the coils of small intestine and the sigmoid colon, as well as between the transverse colon and the liver.

The spleen contained several white infarcts, varying in size from 0.5 to 1 cm. in diameter.

The stomach contained 200 cc. of thin fluid containing coffee-ground-like material. Four ulcers were found on its lesser curvature, the largest estimated to be 3.5 by 2 cm. The other ulcers were 5 to 6 mm. in diameter. In the center of the large ulcer was found an open blood vessel. It was about 0.5 mm. in diameter and was opened for a length of 2 mm.

The liver was estimated to weigh 2000 gm. The surface was bluish red. It contained numerous, irregularly shaped depressed areas, and one light brown, soft area in the dome about 2 cm. in diameter. On section the liver showed numerous infarcts of different sizes and shapes. One large infarct was red and the vessel supplying this region was closed by a small red plug. Most of the other infarcts were white but some contained a thick brownish fluid.

The right pleural sac contained about 100 cc. of dark, thin fluid; the left contained no excess of fluid. There were many firm adhesions between the lungs and the diaphragm.

The pericardial lining was smooth and shining. The sac contained about 20 cc. of blood-stained fluid. There were numerous nodules along the vessels in the heart wall. These were hard, whitish in color and were surrounded by small red zones. There was no embolus in the pulmonary artery. The heart muscle appeared light red in color; that of the left ventricle appeared somewhat thicker than normal. The estimated weight of the heart was 400 gm. The mural and valvular endocardium appeared normal.

On section the lungs showed a slight edema but were otherwise normal.

The kidney capsules stripped easily. Numerous yellowish spots were scattered over the surfaces of both kidneys. On section the right kidney showed numerous infarcts, most of which were white. Some showed reddish zones about the necrotic centers. The left kidney was surrounded by a very large hematoma extending from the diaphragm to the pelvic brim and from the lateral abdominal wall over the midline. This hematoma was entirely within the kidney capsule. On the anterior surface of this kidney was a perforation in the cortex measuring about 5 mm. in diameter which led into a small blood-filled cavity in the cortex. Numerous white infarcts were present in this kidney.

The aorta was smooth throughout its length. The adrenals were not examined.

Anatomical Diagnoses: Periarteritis nodosa; multiple infarcts of kidneys, spleen, liver and stomach wall; hemorrhage from left kidney; hemorrhage from ulcer of stomach.

MICROSCOPIC EXAMINATION

Microscopic examination of the kidneys, liver, heart, intestines and spleen reveals a marked involvement of the small arteries. These vessels all show infiltration of the adventitia with polymorphonuclear leukocytes and small lymphocytes. There is a marked hyaline degeneration of the media and a slight proliferation of the intima. Thrombi are present in many of the larger arteries of the kidneys, spleen and liver. A few of the vessels involved, particularly in the spleen and heart, show a marked fibrous proliferation in both the intima and media, and to a lesser degree in the adventitia. In the intestines many areas of necrosis of the mucosa and submucosa can be seen related to the thrombosed vessels. The spleen, kidney and liver all show areas of infarction. In the liver there is complete necrosis of all the tissue elements in the infarcted areas (Fig. 4). These represent true, so-called anemic infarcts of the liver.

CASE 2. The patient, a white male, aged 24 years, was evidently well until about November, 1933, when he had an attack of what was called "influenza." He never quite recovered from this attack. He felt weak and began to lose weight.

In February, 1934, a gastro-intestinal barium study disclosed a stenosing lesion at the pyloric end of the stomach, evidently carcinoma. He then developed some jaundice and began to have occult blood in the stools. The hemoglobin fell to 18 per cent. Transfusion was performed and the hemoglobin rose to 40 per cent, at which level it stayed to the end, despite constant slight bleeding into the bowel. He developed severe abdominal pains and attacks of vomiting, both of which were controlled to some degree by pantopon. The blood pressure was 130/80. The heart was normal, the lungs were clear. The liver seemed enlarged and irregular. The spleen was not palpable. There was constantly moderate to marked distention of the abdomen. The patient was put on a liquid diet but terminally could retain nothing taken by mouth but Vichy water.

During the last 2½ weeks of life he ran an elevated temperature, up to 101.5° F. The chest remained clear. The jaundice became progressively more intense.

The urine upon one occasion showed a specific gravity of 1024, acid reaction, trace of albumin, negative sugar, occasional red and white cells, 4 plus bilirubin, but no urobilinogen. The stools constantly showed 4 plus occult blood but were never frankly tarry. The patient evidently died of inanition.

AUTOPSY REPORT

The body was well developed but extremely emaciated. No edema or cyanosis; jaundice Grade 4 of the skin and mucous membranes. A hard nodular mass could be palpated in the epigastrium, extending down to within 3 cm. of the umbilicus in the midline; it was evidently not the liver. The autopsy was limited to the abdomen.

About 2000 cc. of clear bile-stained fluid was present in the peritoneal cavity. The nodular mass proved to be a large tumor attached to the greater curvature of the stomach at its pyloric end. The liver was at the rib margin. The appendix was normal. The diaphragm was at the fourth interspace on each side.

The spleen weighed 300 gm. There were several infarcts about 1 cm. in diameter situated just under the capsule. The spleen was congested and was somewhat firmer than normal.

The liver weighed about 1500 gm. It was deep olive green in color and its surface was slightly nodular. On section the elevations proved to be small yellowish areas, which were found to be infarcts (Fig. 1). Greenish, clear bile flowed from the surface of the liver. The gall-bladder, the cystic duct and both hepatic ducts were much dilated. The common bile duct and the hepatic artery were compressed to the point of complete closure by a mass of tumor continuous with that in the stomach (Fig. 2). The anterior wall of the portal vein was infiltrated with tumor but its lumen was patent.

The rugae of the stomach were hypertrophied and the stomach was coated with a layer of fresh blood. At the pyloric end was an ulcer 6 cm. in diameter with a necrotic base and with hard elevated borders. The tumor had penetrated the stomach wall and had infiltrated the head of the pancreas as well as the lesser omentum. It had grown down along the common bile duct and around the ampulla of Vater, which measured 1 by 0.5 cm. and protruded into the lumen of the duodenum (Fig. 3). The rest of the gastro-intestinal tract was normal.

The pancreas was extensively infiltrated by tumor. The parts not involved were firm and fibrous.

The right kidney weighed 125 gm., the left 150 gm. On section they were bile-stained but otherwise normal. The urinary bladder and genital organs were normal.

The aorta was normal. There was a large mass of periaortic lymph nodes, the individual nodes varying from 1 to 2 cm. in diameter.

The head was not examined.

Anatomical Diagnoses: Carcinoma of the pyloric end of the stomach with metastases to the pancreas, periaortic lymph nodes and lesser omentum; obstruction of the common bile duct and hepatic artery; ascites; severe inanition; jaundice; multiple infarcts of the liver and spleen.

MICROSCOPIC EXAMINATION

Microscopically the tumor of the stomach proves to be a scirrhous carcinoma. The liver shows multiple areas of infarction. These each include from one to several liver lobules. In these areas there is a complete necrosis of the blood vessels and of the connective tissue septa as well as of the liver cords (Fig. 4). No evidence of a predilection for any particular zone of the liver lobule can be observed. Occasional portal venules both within and at the periphery of necrotic areas are thrombosed. The necrotic areas merge gradually at their peripheries with the normal liver tissue. The infarcted areas are surrounded by a few polymorphonuclear and mononuclear cells, but on the whole there is very little cellular reaction to the dead tissue. The peripheral parts of the infarcts are bile-stained. The rest of the liver tissue shows slight atrophy of the liver cords and marked stasis of bile, particularly in the form of bile thrombi.

Because of the unexplained infarcts of the spleen we must admit that, although the occlusion of the hepatic artery seems sufficient to account for the infarction of the liver, a mural thrombus may have been present or a subterminal endocarditis which cast off emboli producing the infarcts in the liver as well as in the spleen. However, because of the lack of embolic phenomena elsewhere and because of the large number of infarcts in the liver we are inclined to discount this possibility.

SUMMARY

A review of the literature of infarction of the liver with a report of 2 additional cases is given.

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DESCRIPTION OF PLATE

PLATE 68

- FIG. 1. Case 2. Gross appearance of the infarcts of the liver.
- FIG. 2. Case 2. Section through the free margin of the lesser omentum. The arrow points to the hepatic artery surrounded and compressed by a large mass of tumor tissue.
- FIG. 3. Case 2. Gross appearance of the carcinoma of the pyloric end of the stomach.
- FIG. 4. Case 2. Low power view of an infarct of the liver. Note the nearly complete disintegration of the portal canals as well as of the liver cords in the infarcted area. Similar lesions were abundant in Case 1.



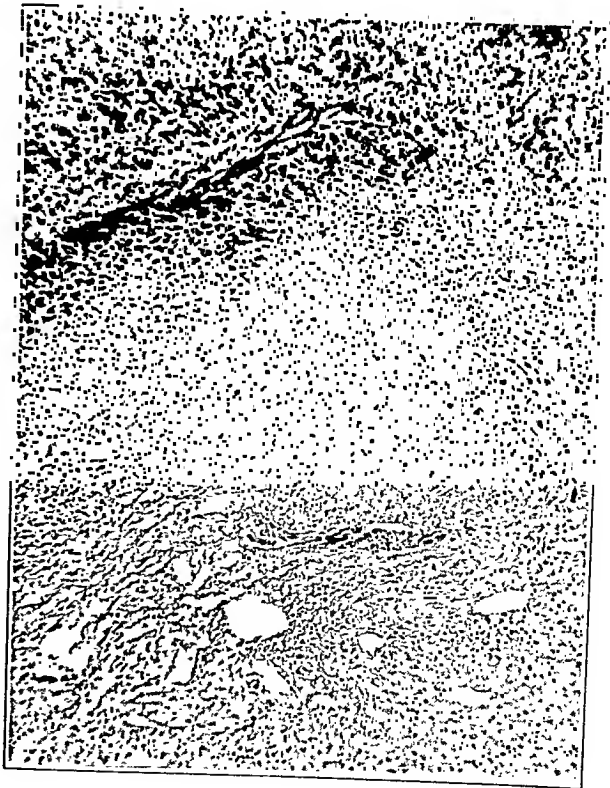
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Infarction of the Liver

PRIMARY ADENOCARCINOMA OF THE PANCREAS IN A FIFTEEN YEAR OLD BOY *

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REVIEW OF THE LITERATURE

Primary carcinoma of the pancreas in children is rare. Extensive search of the literature revealed the report of relatively few authentic cases. Of those that have been found some were unproved and others were doubtful cases. Inadequate description, incomplete postmortem examinations, use of vague equivocal terms such as "cancer-like induration," absence of microscopic study and confusing case histories make the analysis of these cases difficult. The proved reported cases of primary carcinoma of the pancreas in persons under 20 years of age in the literature are 5 in number.

CASE 1. Bohn,¹ 1885, a 7 months old female infant. The tumor arose in the head of the pancreas with metastases to surrounding lymph nodes and liver. Cytological examination revealed a typical carcinoma simplex.

CASE 2. Kühn,² 1887, a 2 year old girl. The head of the pancreas was involved by tumor, as were the neighboring lymph nodes, liver and lungs. Microscopically the tumor was an adenoid cylindrical cell carcinoma.

CASE 3. Simon,³ 1889, a 13 year old boy. The tumor was "two-fist" sized, involving the head of the pancreas and infiltrating through the mucosa of the duodenum. The tail of the pancreas was relatively uninvolved. The bile ducts were compressed by the tumor with dilatation above. Metastases to liver, surrounding lymph nodes and kidneys were present. No microscopic study was reported.

CASE 4. Kaufmann,⁴ 1929, a 19 year old girl. The tumor, which was the size of a bean, arose in the tail of the pancreas, with numerous metastatic nodules in the liver (3300 gm.) and carcinomatosis of the pulmonary lymphatics. Microscopically this was a carcinoma solidum with medium sized cells.

* Received for publication October 22, 1934.

CASE 5. Stout and Todd,⁵ 1932, a 4 year old boy. The head of the pancreas was enlarged and increased in density. The tumor was about 5 cm. in diameter, unencapsulated. It encroached upon but did not involve the duodenum. The neighboring lymph nodes and the liver showed metastases. Microscopically the tumor was an adenocarcinoma, probably arising from the ductal epithelium.

Schamoni,⁶ in 1924, mentioned a case of carcinoma of the pancreas in a 16 year old boy. The case was not reported as such but was listed in a statistical study of malignant tumors before and after the World War. No description was given.

Gruber⁷ and Merkel⁸ cited von Sotow's⁹ case in a 1½ year old child, but despite extensive efforts the original case report could not be obtained.

In 1915 Stewart and Stewart¹⁰ reported a carcinoma of the tail of the pancreas in a 9 year old boy. An autopsy, limited to the surgical incision only, was permitted and the data submitted do not allow conclusions as to whether this was a primary or secondary tumor of the pancreas.

Philipp¹¹ cited cases by Todd, Battersby, Hofmann and von Rokitsansky as doubtful, because of inadequate description and because they dated back to times when knowledge of the diseases of the pancreas was unsatisfactory.

In 1818 Todd¹² reported a case of a 14 year old girl who complained of severe upper abdominal pain, with icterus, emaciation and edema of the lower extremities. At autopsy the pancreas was "scirrhus" and the head and surrounding tissues formed a hard solid mass which entirely obliterated the lumen of the distal end of the common bile duct. The proximal portion of the duct formed a huge dilated sac extending from the porta hepatis to the os sacrum. The gall-bladder was not dilated because of a kinking of the cystic duct. No mention was made of metastases. Battersby's¹³ case in a 14 year old girl was found to be a reference to Todd's case.

In 1866 Hofmann¹⁴ reported a case in an 8 months old premature infant who lived only a half hour. The placenta was grossly normal. The pancreas was very hard and nodular. The mesenteric lymph nodes were enlarged and nodules were present in the liver. Professor Wedl called this a carcinoma arising in the pancreas. However, the mother had had numerous stillborn children from no apparent

cause. This case was considered doubtful because of the history of probable syphilis and the lack of microscopic confirmation.

In 1842 von Rokitsky¹⁵ mentioned a case of cancerous infiltration of the entire pancreas of a newborn infant, reported by Berg during his stay at von Rokitsky's clinic.

Gruber⁷ cited Dutil¹⁶ as having reported a case in 1888 in a 14 year old child, but examination of the original reference revealed the patient to be a 47 year old coach driver.

Oser¹⁷ and Rauschmann¹⁸ attributed a case of primary carcinoma of the pancreas in a 13 year old boy to Bandelier¹⁹ in 1896. However, the case was identical with the one described by Simon, and no doubt was the same case.

Williams²⁰ stated Israel²¹ reported a case in a 13 year old child but this was a carcinoma of the kidney.

Claessen²² mentioned a case report by Harder of a 14 year old girl in whom the pancreas was found to be of scirrhus hardness and, in the region of the liver, to be of fist-size or larger. On sectioning the pancreas, purulent material flowed out. Abscesses were present in the right lung. Claessen²² and Wolff²³ thought this case to be extremely doubtful.

Rauschmann¹⁸ cited a case by Cruveilhier²⁴ in a stillborn male infant and Williams²⁰ listed Arnozan²⁵ as reporting a "so-called congenital cancer." The original articles could not be found.

A sixth proved case of primary carcinoma of the pancreas in an individual under 20 years of age is reported.

REPORT OF CASE

Clinical History: J. S., a boy, 15 years of age, of American parentage, was admitted to the medical service of Cleveland City Hospital, Nov. 4, 1930, complaining of icterus, increasing lassitude and loss of weight. The present illness dated back 5 or 6 months and began with general malaise. During this time he had had numerous attacks of "summer complaint," in which he had had considerable gas and sour, bitter eructations. The jaundice, first noted 3 months prior to admission, had cleared up under a physician's care and frequent doses of castor oil, but had reappeared in 2 to 3 weeks without much change in subjective feeling. Pruritis had been mild. Fatty and sweet foods had been excluded from his diet. He had noticed a few light-colored stools, and the urine had been unusually dark. The patient had lost 15 or 20 pounds in the last 3 months.

Physical Examination: The patient was well developed, well nourished, intelligent and coöperative. The skin and sclerae were markedly icteric; the tonsils were subacutely inflamed. Heart and lungs presented no abnormality. The

liver was slightly tender and enlarged. Other than a phimosis no additional abnormal physical finding was noted.

Laboratory Studies: A persistent uribilinuria and an icteric index ranging from 38 to 60 was revealed. The red blood count was 4,810,000 and the hemoglobin was 92 per cent Sahli. The white blood count fluctuated between 14,000 and 22,000. Differential count revealed an eosinophilia which varied from 21 to 7 per cent. The red blood cells showed an increased fragility to hypotonic saline solutions. The clotting time was 15 minutes by Sabarze's method, as contrasted to 3 to 4 minutes as normal for that method. The bleeding time was 5½ to 6 minutes, the normal being 1 to 3 minutes. The stools contained no bile pigment and no parasites or ova. The blood sugar was 86 mg. per 100 cc.

The temperature fluctuated between 37° and 39° C. The respirations varied between 15 and 24 per minute, the pulse between 58 and 120 beats per minute.

Course of Illness: On entry a diagnosis of acute catarrhal jaundice was made but the lack of improvement and persistent eosinophilia suggested intestinal parasitism with obstruction of the common bile duct. An exploratory laparotomy was advised but refused by the parents. The patient was discharged on release, Dec. 27, 1930.

He felt a trifle better and was up and about for 4 weeks until he experienced pain in the region of the liver. This increased in intensity and a severe colicky pain developed in the right upper quadrant with radiation to the back. After 2 weeks in bed he was up and about again but had tenderness and cramps in the upper abdomen. He lost weight progressively and his general condition became worse. The icterus deepened. The patient gradually became weaker and more lethargic until he lapsed into semicoma and was admitted on the surgical service of St. Vincent's Charity Hospital on March 23, 1931.

On admission physical examination revealed marked emaciation, icterus and a tender enlarged liver. No masses were palpable in the abdomen. The patient was semicomatose and apparently moribund. That night, intractable hemorrhage from the left nostril developed and, despite all treatment, persisted until the patient died, 14 hours after admission.

AUTOPSY REPORT

Autopsy was performed 2 hours after death. The abdominal cavity contained about 100 cc. of dark, amber-colored fluid. The liver was markedly enlarged. A hard, irregular, rounded nodular mass about 7 cm. in diameter was present in the head of the pancreas and presented a mottled red, blue and yellowish white color. On section the mass showed a variegated, coarsely trabeculated cut surface. The tumor was granular, yellow to yellowish white in color, with irregular hemorrhagic discolorations of brownish red. About the periphery were numerous, enlarged firm lymph nodes, which on section showed the same yellow and white, granular, highly cellular cut surfaces. The rest of the pancreas was definitely atrophic, firm in consistence, with marked increase of interlobar connective tissue stroma. The pancreatic duct was moderately dilated.

The gall-bladder was greatly distended, as was the entire biliary tract. No bile could be expressed into the duodenum except after cutting through the tumor mass and freeing the common bile duct. On opening the duct system the dilatation was found to start 1 cm. from the ampulla of Vater and to extend into the intrahepatic ducts. The biliary obstruction was caused by compression of the common bile duct by the tumor mass, which did not extend into the wall of the duct or the duodenum. The liver weighed 3800 gm. and was diffusely and symmetrically enlarged. Numerous, rounded, yellowish white nodules characterized the capsular and cut surfaces, and the intervening hepatic tissue was a mottled green and brown color. The lobules were abnormally prominent and the perilobular stroma was increased in amount. No metastases other than to the liver and neighboring lymph nodes were found.

MICROSCOPIC EXAMINATION

Microscopic sections through the pancreatic tumor exhibit irregular masses and interlacing cords of cells with a slight tendency to form acini. These are circumscribed by dense, hyalinized connective tissue. Areas of necrosis and extensive hemorrhage are prominent. Instances of tumor invasion of veins are numerous. The cells vary considerably in size and in form, from cylindrical to spindle shape. The cytoplasm is acidophilic. The nuclei are large, vesicular and situated in the lower portion of the cylindrical cells. Nucleoli are prominent. Numerous normal and a few abnormal mitotic figures are seen. Sections through the rest of the pancreas reveal a marked increase in inter- and intralobular connective tissue, atrophy of the acinous parenchyma and remarkable preservation of the islets of Langerhans. The secretory acini show great variation from closely packed, highly cellular, normal appearing to small, irregular, deeply staining, widely separated acini. Some of the latter show a close resemblance to the tumor tissue.

Sections of the liver reveal early obstructive biliary cirrhosis, with increase of stroma, deposition and phagocytosis of bile pigment, dilatation of sinusoids and bile ducts and an apparent increase in number of periportal bile canaliculi. The tumor metastases show cell groups which tend to assume rounded and rosette-like shapes, for the most part without lumens. Some of the periportal lymphatics and veins contain tumor masses.

The rest of the organs show nothing striking, other than hemorrhage into the nasopharynx, tracheobronchial tree and pelvis of the right kidney. There is a marked, acute pulmonary distention.

The immediate cause of death was asphyxia from hemorrhage into the tracheobronchial tree.

SUMMARY

1. The literature on primary carcinoma of the pancreas in individuals under 20 years of age is reviewed critically.

2. Five proved, reported cases of primary carcinoma of the pancreas in subjects under 20 years of age were found.

3. A sixth case, that of an adenocarcinoma of the pancreas in a 15 year old boy, is reported. The symptomatology and course of the disease were typical of carcinoma of the head of the pancreas with biliary obstruction. Death was due to asphyxia from hemorrhage into the tracheobronchial tree.

4. Two other references to primary carcinoma of the pancreas in children were found. One was unobtainable and the other merely cited a case without giving details.

5. Five other case reports of primary carcinoma of the pancreas in children were found, analyzed and listed as doubtful cases.

NOTE: I am indebted to Dr. H. T. Karsner for many helpful suggestions and criticisms, and to Dr. Paul Gross, pathologist at St. Vincent's Charity Hospital, for aid in the preparation of this paper.

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BASOPHILIC DEGENERATION OF HEART MUSCLE *

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In 1910, Hewitt,¹ as a result of a study in the Department of Pathology of the University of Minnesota, published a paper entitled, "A Peculiar Degeneration Found in Heart Muscle Cells." Therein he described the lesion as "a small, round, oval, or irregular pale blue area inside of a single muscle cell." He continued, "With high power these degenerations show a slight bluish mottling, somewhat irregularly defined. . . . They sometimes occupy only a portion of the cell, at other times almost the whole of the cell is filled with blue staining material, but always enough of the cell remains to show that it is a heart-muscle cell." As far as the literature has been reviewed, there is no other reference to this type of lesion.

My investigation is based on a study of routinely obtained sections of the heart derived from 320 consecutive postmortem examinations. Blocks of tissue were taken from the septum and both ventricles of each heart that was studied; these were hardened in Orth's fixative and embedded in paraffin. The sections were then stained in the routine manner with hematoxylin and eosin, and in addition with various other stains which will be discussed later.

The lesion described by Hewitt was found in 107, or 33.43 per cent of the 320 hearts examined. The areas of degeneration were of microscopic size. It is my impression that the degeneration passes through several phases. With the hematoxylin and eosin stain, the degenerated portions were seen as basophilic patches which generally occupied the centers of the muscle bundles and frequently included the well preserved, often hypertrophied, nucleus. They always were of well defined limits and at the periphery of each was a zone of normal muscle tissue. No tissue reaction could be seen in the degenerated areas. In the immediate neighborhood, and even in the continuation of the involved muscle bundle, the normal structure was preserved. No gross change in the heart muscle could be observed.

In the early stage of the process these degenerated areas stained a rather dark blue, were fairly dense, finely granular, occasionally

* Received for publication October 26, 1934.

clumped, and sometimes included fragments of muscle fibers (Fig. 1). As the process advanced these areas stained a lighter blue and what was apparently vacuolization appeared (Figs. 2 and 3). In very advanced stages there was left only a fine network of basophilic, intertwining fibers, which included small tissue spaces (Figs. 4 and 5). In this stage the lesion was rather indistinct. If the lesion were very small, or if the heart muscle were cut transversely, the areas were difficult to find; otherwise they were easily distinguished. They might be scattered diffusely throughout the tissue and only a few could be found, and at other times they might be numerous.

The most frequent single site of the lesion (Table I) was the septum. Closely following this in frequency was the left ventricle; the right ventricle showed fewer lesions. Of the cases in which the lesion was found in more than one site, those in which the septum and left ventricle were involved were the most numerous. It is interesting to note that the lesion was found only five times in the right ventricle alone, and was found in combination in the right ventricle and septum only twice.

There was no significant difference in incidence as to sex. Of the 320 hearts, 204 were from males and 116 from females. The regions of degeneration were found in 70 hearts of males and in 37 hearts of females, or in a total of 107 of the entire 320 hearts.

Considering the incidence of the lesion, it is astonishing that only the preliminary report by Hewitt has been found in the entire literature, as far as it has been reviewed. Hewitt presented 2 cases of basophilic degeneration of the heart muscle which he had found in routine examination of sections of heart. The number of hearts he examined is not given, but his study covered 6 months. In my series of cases basophilic degeneration was found in about a third of the hearts examined routinely.

Hewitt stated that this peculiar degeneration occurred where there was marked proliferation of connective tissue and where there was evidence of pressure atrophy. In hearts I examined it occurred also where there was no proliferation of connective tissue, and the cells appeared slightly hypertrophied. Occurrence of the degeneration in areas of fibrous degeneration within myocardial scars was rather infrequent. In infarcted hearts basophilic degeneration was not seen in the muscular remnants within the fibrous tissue but was frequently found some distance away.

In order to ascertain the nature of the lesion several differentiating stains were used. With fat stain (scharlach R) the areas showed evidence of affinity for the basic component of the stain and appeared a darker grayish blue than the surrounding tissue. In sections stained for iron noticeable changes in the involved areas were not revealed. With Van Gieson's stain there were no special differentiating characteristics. With the Mallory-Heidenhain method the areas of degeneration showed some affinity for aniline blue, but stained a much lighter blue than the connective tissue. With the Mayer carmine method the areas stained distinctly light red, indicating the

TABLE I
Situation of Lesion

Location	Cases
Septum only	32
Left ventricle only	27
Right ventricle only	5
Septum and left ventricle	33
Septum and right ventricle	2
Septum and right and left ventricles	6
Left ventricle and right ventricle	8
Total	113

presence of some kind of mucin or of a substance related to mucin. Hewitt, however, stated that these areas did not take any characteristic stain for mucin. With Best's carmine method the areas of basophilic degeneration stained bright red and were readily distinguished from the bluish gray of the surrounding tissue. It must be kept in mind that the substance in these areas may be related to glycogen, in spite of the fact that the blocks were kept in formalin. It is worth mentioning that a similarity in staining reaction for mucin and glycogen exists in the areas of basophilic degeneration of the heart and in the corpora amylacea of the various organs. The presence of calcium in these areas has been excluded by the acid test. With the Orlandi silver stain the involved areas appeared very pale and gave evidence of some liquefaction. There was no argentophilic reaction to this stain.

To quote Hewitt, who used iodine in the form of compound solution of iodine (Lugol's solution), "these areas, when so stained and

examined under water, appear of a terra cotta pink, somewhat mottled." Although I carried out the procedures exactly as he described them, I could not obtain this reaction with the materials available to me. Hewitt further described, in sections stained with toluidin blue and thionin, "large granulated cells which often are especially abundant about the blood vessels . . . and which are thought to be the same as described by Lustgarten as the parasite of

TABLE II

Anatomical Diagnoses of Cases in which Basophilic Degeneration was found at Postmortem Examination of the Heart

Diagnosis	Hearts examined	Basophilic degeneration found
Malignancy	81	37
Heart disease	52	21
Cholecystitis with cholelithiasis	20	9
Gastro-intestinal ulcers	16	6
Pneumonia	16	9
Peritonitis	15	5
Brain tumor	21	4
Syphilis	9	4
Genito-urinary infection	7	4
Ulcerative colitis	5	2
Empyema of thorax	3	2
Acute pancreatitis	1	1
Electrocution	1	1
Drowning	1	1
Toxemia from burning	1	1

syphilis." This cellular reaction has apparently no local connection with the areas of basophilic degeneration. The presence of these large granulated cells could not be confirmed in any of my specimens, some of which were derived from patients whose condition was diagnosed as syphilis. Of Hewitt's 2 reported cases, 1 was an example of tertiary syphilis, and the other was a case of acute serofibrinous peritonitis with abscesses in the liver and mesentery.

In Table II is shown the frequency of occurrence of basophilic degeneration in my cases, together with the primary pathological diagnosis. The lesion was found most frequently in cases of malignancy and heart disease. It was also found in many cases of infection and inflammation, as well as in a single case each of electrocution, drowning, and toxemia from burns. Because of the multiplicity of condi-

tions with which basophilic degeneration has been found to be associated, it would be difficult to give any one disease entity as the etiological agent.

From Table III it may be seen that the lesion occurs frequently among subjects who have passed the age of 30 years and most frequently among those who are between the ages of 40 and 80 years. Malignancy and a diseased condition of the heart, in association with

TABLE III
Age Incidence

Decade of life	Hearts examined	Basophilic degeneration found	
		Number	Per cent
yrs.			
0-10.....	27	0	
10-20.....	8	1	12.50
20-30.....	17	1	5.88
30-40.....	40	9	22.50
40-50.....	43	16	37.20
50-60.....	70	29	41.42
60-70.....	73	31	42.46
70-80.....	39	19	48.70
80-90.....	3	1	33.33
Total	320	107	33.43

which the lesion was most frequently seen (Table II), generally afflict persons who are 40 to 80 years of age; thus, age seems to be an important, although probably not the only, factor concerned. The factor of toxemia cannot be ignored.

Hewitt quoted Mallory as follows: "The nature of this degeneration is of a hyaline change allied to hydropic degeneration with the presence of some mucin." In an article by Rademaker,² which deals with lesions caused by sarcosporidia in the heart muscle, there is a photomicrograph of a stage of the lesion which resembles slightly the picture of the early stage of basophilic degeneration. The change pictured by Rademaker, however, does not correspond in staining reaction to the areas under consideration. No sign or manifestation of parasites could be observed in the great number of sections studied. Because of the strict localization of the lesions, the possibility has to be considered that they are of parasitic origin, but since parasites were not found this possibility does not seem to me to be tenable.

SUMMARY AND CONCLUSIONS

A peculiar lesion of the heart muscle is described under the term "basophilic degeneration." One subject, in whose heart the lesion was found, had died as early as the 17th year of life, but most of the hearts were from subjects who had died between the ages of 40 and 80 years.

The most frequent sites of the lesion were the septum, the left ventricle, and a combination of the septum and the left ventricle.

Staining reactions showed that the areas contained mucin as well as a component related to glycogen.

It seems probable that toxemia played some part in the etiology of this lesion.

Hematoxylin and eosin was the most valuable stain, because it clearly differentiated the areas of basophilic degeneration.

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DESCRIPTION OF PLATE

PLATE 69

- FIG. 1. Transverse section. The area of degeneration is fairly large, granular, and stains bluish purple. The nucleus is well preserved. Hematoxylin and eosin. $\times 700$.
- FIG. 2. Area of basophilic degeneration in a more advanced stage than is shown in Fig. 1. The periphery of the muscle cell is intact. Hematoxylin and eosin. $\times 200$.
- FIG. 3. Higher magnification of a portion of the section shown in Fig. 2. $\times 550$.
- FIG. 4. A fairly well advanced stage of basophilic degeneration. Fibers which stained pale blue are seen surrounding remnants of muscle fibers. $\times 200$.
- FIG. 5. Higher power of a portion of the section shown in Fig. 4. The area appears distinctly vacuolated. $\times 550$.



1



2



3



4



5

Haumeder

Basophilic Degeneration of Heart Muscle

ADENO-ACANTHOMA OF THE PYLORUS *

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Adeno-acanthoma of the pylorus is practically unknown and yet carcinoma of the stomach is a very common condition, 60 per cent of all gastric carcinomas originating in the pyloric region (Ewing,¹ and Kaufmann²). Thousands of cases have been carefully studied, so that there has been no dearth of material in which to observe the incidence of squamous cell carcinoma in the pylorus. But 3 cases of adeno-acanthoma of the pylorus are recorded in the literature, namely, those of Lubarsch³ in 1906, Herxheimer⁴ in 1907, who first suggested the designation "adeno-acanthoma" for mixed epidermoid and glandular pyloric carcinomas, and Oberling and Wolf⁵ in 1927.

Lubarsch³ reported an adenocarcinoma of the pylorus which had metastasized to the regional lymph nodes. In these metastases he found numerous foci of squamous cell tumor. On reëxamination of the primary tumor in the pylorus he discovered a single area showing typical cornifying prickle cell epithelioma which looked "precisely like cancer of the esophagus."

In Herxheimer's case,⁴ at operation a tumor the size of a child's fist was found encircling the pylorus. Miliary nodules studded the serous surface and two enlarged lymph nodes were found behind the pylorus. The remainder of the stomach was tumor-free. The patient died 8 days after operation. At autopsy no metastases or extensions were found and there was no local recurrence.

Histologically the tumor was predominantly an adenocarcinoma. A comparatively small part of the tumor was epidermoid in character. All gradations between adenocarcinoma and prickle cell epithelioma were demonstrable. Pearl body formation was common in the epidermoid areas. Epidermoid masses were especially common in the submucosa and deeper parts of the tumor down to the serosa. The connective tissue between the tumor cell masses contained a moderate number of elastic fibers. Irregular infiltration by round cells was present throughout the tumor.

* Received for publication November 10, 1934.

The case reported by Oberling and Wolf⁵ was that of a female, 67 years of age. Exploratory laparotomy disclosed the parietal peritoneum covered with whitish, often hemorrhagic nodules. The abdominal viscera were adherent and covered with disseminated nodules. At autopsy the neoplastic process was limited to the abdomen. The abdominal viscera were found to be entirely covered with globular masses, reddish to purplish in color and of a gelatinous consistence. A huge, hemorrhagic gelatinous mass weighing 11 Kg. involved the omentum. The stomach was about normal in volume. At the level of the pylorus the gelatinous masses in the serosa were in direct continuity with a homogeneous, whitish infiltration which occupied all layers of the wall and formed an irregular, ulcerated elevation on the mucosa. The liver was seeded with semiliquid viscous nodules up to the size of a man's fist. A nodule of similar appearance was found in the spleen.

Histological examination disclosed a mucous adenocarcinoma of the stomach which in areas presented an intracystic papillary structure lined by clear cells ("analogous with certain renal tumors with clear cells"). In the midst of these glandular elements large cylinders of prickle cell epithelium exhibiting large pearl bodies were found. The authors remarked that the picture was strikingly like cutaneous epithelioma. Adenocarcinoma and epidermoid carcinoma were more or less intermingled, but transition forms between cylindrical and squamous elements were not demonstrable in a study of many sections. The epidermoid formation was confined to the central portion of the glandular tumor. The extragastric tumor was a mucous adenocarcinoma and the metastases were entirely mucoid.

REPORT OF CASE

Clinical History: * P. M. entered the hospital, on March 9, 1934. He was a seaman, aged 48 years, and was born in Norway. His father and mother were living and well at the age of 70 years. Three brothers and five sisters were living and well. The patient was the oldest child.

His chief complaint on entering the hospital was pain in the abdomen and vomiting. Prior to Christmas of 1933, his health had been good. At that time his appetite gradually became poor, he was troubled with constipation, belching and epigastric discomfort. About the same time he began vomiting variable amounts of "coffee-ground" material on an average of every 3 or 4 days,

* The clinical and operative summaries were prepared by Drs. Teufel and Van Ackeren of the U. S. Marine Hospital at Seattle, Washington.

and often had black, tarry looking stools. He grew progressively weaker and lost about 20 pounds in weight up to the date of admission. He also became paler than formerly.

Laboratory Data: The blood Wassermann examination was negative, the blood count was 3,300,000 on March 12th, and 3,070,000 on March 21st. The hemoglobin ran 65 to 70 per cent and the stools repeatedly showed occult blood 4 plus. X-ray examination indicated a carcinoma of the pylorus with almost total pyloric obstruction.

Operation: The patient was transfused on March 30th and operated upon on April 2nd. A tumor of the pylorus about 8 cm. in diameter was found. It was not adherent. During the operation, when the stomach was bisected, the large opening gave ample opportunity to examine the proximal end both by inspection and by palpation. There was no evidence of carcinoma. There were no evident metastases in other viscera and enlarged lymph nodes were not found. The tumor was so well confined to the pyloric portion of the stomach that after partial gastrectomy the surgeon felt hopeful of recovery.

After the operation the patient gradually gained weight and strength and the wound healed. Early in May he complained of pain in the operative scar. Palpation suggested a mass in the epigastrium to the right of the scar. On May 12th symptoms of obstruction supervened. The patient was kept under a palliative regimen, but continued to grow worse and died on July 11th.

Surgical Specimen: The formalin-fixed specimen represented the pyloric portion of the stomach and a short segment of the duodenum. The outward appearance was that of a more or less smooth, bulging mass showing fibrous thickening of the covering surface. Dissection of the specimen disclosed two small lymph nodes along the posterior inferior surface. A section through the most prominent portion of the mass revealed an ulcerated, vegetating, more or less cornifying structure protruding into the lumen. The growth gradually tapered off all around into a hard, leathery mucosa, the normal markings of which were ironed out for variable distances before merging with the normally folded and furrowed mucosa. The tumor measured 9 by 6 by 4 cm. The cut surface presented a mosaic of whitish and yellowish, roughly rounded and angular, solid, sharply demarcated masses up to 16 mm. in longest diameter, varying from white to golden yellow in color. These infiltrated through to the serosal surface. Near the distal end of the pylorus they were readily discernible in the submucosa, blended for some distance with the mucosa and stood in sharp contrast to the thick muscularis below, which in this portion appeared intact in gross.

AUTOPSY REPORT

The autopsy disclosed massive local extension of the neoplastic process centering from the line of the anastomosis. This mass was adherent to the abdominal wall, the pancreas and the celiac vessels. The omentum was included. The portion of jejunum which had been anastomosed to the stomach was infiltrated for a distance of about 2 inches by a hard leathery growth, which apparently completely encircled it. There were no distant metastases.

Careful dissection of the formalin-fixed specimen disclosed the following. The pars abdominalis of the esophagus, and the cardia of the stomach appeared normal. Below this level the stomach wall showed progressive thickening and more or less suppression of the mucosal plication progressing downward to complete obliteration. In the line of the gastrectomy scar and in the vicinity of the anastomosis the wall reached a thickness of 11 mm. and was so indurated that it cut like cartilage. Here the gastric surface looked and felt like alligator skin, while the sectioned wall presented a ligamentous appearance. The left adrenal gland and a large piece of the body of the pancreas were firmly adherent to the fibrosed gastric serosa. Several medium sized, sclerosed arteries traversed this entangled mass.

HISTOLOGICAL EXAMINATION

Most of the material studied was impregnated for 48 hours in 2½ per cent potassium bichromate after formalin fixation. In all, thirty-nine blocks were taken from different areas of the surgical specimen and thirty-two from the autopsy material. A number of 43 by 70 mm. paraffin sections were made in order to afford every opportunity for observing transitional and other noteworthy changes. The sections were stained with Weigert's iron chloride hematoxylin and Van Gieson's picro-fuchsin, Mayer's acid hemalum and eosin, a modification of the Romanowsky stain for the demonstration of intercellular bridges, Gram's stain for keratohyaline granules, toluidin blue for mucin, and Weigert's resorcin-fuchsin for elastic fibrils.

The tumor removed at operation is predominantly epidermoid carcinoma. The mass that protruded into the lumen is the counterpart of a cornifying papillary and infiltrating prickly cell epithelioma

of the skin. The normal architecture of the pyloric wall is entirely obliterated by solid, bulky, angular, lobular and elongated masses of squamous epithelium. Keratohyaline granules, cytoplasmic hyalinization, and cornification in all stages are readily demonstrable. Here and there are seen cysts filled with exfoliated squames. The nuclei are large, round and hyperchromatic and show more or less variation in size and density from area to area. Mitoses are numerous. Scattered mono- and multinucleated, squamous, epithelial tumor giant cells are encountered alone, and in epithelial islands, some reaching a diameter of 120μ . Squamous tumor extends through to the serosa. The stroma is scanty, fibroblastic in character and shows small foci of infiltration by lymphocytes. Ramifying elastic fibers are fairly numerous. The intragastric surface shows stretches of ulceration and granulation and heavy deposits of bacteria. It is interesting to note that the epithelium bordering the ulcers is distinctly anaplastic in character and disposed in branching strands which fray out into the stroma.

Most of the sections show only epidermoid carcinoma. A few sections show sporadically disposed solitary glands and small collections of glands lined by medium and tall columnar epithelium with disorderly arranged hyperchromatic nuclei and goblet formation. Within some glands a few swollen signet ring cells are embedded in mucus.

Sections taken near the margins of the bulky mass show increasing numbers of glandular structures, a partly glandular mucosa, and some persistent landmarks of the gastric wall.

In passing from normal to indurated stomach wall there are seen successively glandular hypertrophy, marked vascular engorgement, mucous hypersecretion, accentuation of the stroma, and heavy infiltrations of lymphocytes. Occasionally the glands form mucous cysts. Here and there the lymphoid follicles are greatly enlarged, the submucosa shows a heavy overgrowth of connective tissue and the muscularis also shows ramifying strands of fibrous tissue. The glands become more and more elongated, and apparently normal glands penetrate here and there through the muscularis mucosa and submucosa. Abruptly, the mucosa and penetrating glands present an atypical and hyperchromatic appearance and all layers of the stomach are irregularly infiltrated by orderly and disorderly glands of variable size and shape, lined by medium and high columnar hyper-

chromatic epithelium showing mucus formation and mucous cysts. Scattered intracystic papillary proliferations are found and in these areas the glandular epithelium becomes distinctly of the clear cell type. Copious collections of swollen, mucus-filled signet ring cells are encountered lying loose in the stroma and within glands. Nerves are commonly infiltrated by tumor tissue, occasionally to the point of obliteration. Tumor thrombi are numerous in the submucosal and subserosal vessels. They are invariably of the cylindrical or mucous signet ring cell type. Stretches of hemorrhage and necrosis are encountered throughout the glandular portion of the tumor.

Sections from some indurated areas, in the vicinity of the major tumor mass, show a chronic productive and suppurating process involving the submucosa and muscularis. The mucosal and infiltrating glands appear more anaplastic and the lining epithelium is frequently stratified. Here and there the columnar epithelium becomes cubical and flattened, roughly polygonal cells are seen superimposed upon or interpolated between the cylindrical cells. Elsewhere portions of glands forming mucus show proliferation of pavement epithelium into the lumen, and in some glands the cylindrical epithelium continues insensibly into the peripheral columnar layer of a squamous patch. Plugs of cornifying epithelium are not uncommonly seen in otherwise well formed mucous glands. In some glands the epithelium shows a progressive heaping up of pavement cells, producing tubules lined partly by squamous epithelium and partly by mucus-forming cylindrical epithelium. Here and there stretches of the mucosa show patches of superficially hyperkeratotic, sharply papillary, stratified squamous epithelium grading insensibly into the surrounding adenocarcinomatous mucosa. Other portions of the mucosa show abrupt transitions from adenocarcinoma to epidermoid carcinoma. Sporadic neoplastic glands remain in the stroma of epidermoid areas.

The transitional phases of adenocarcinoma to squamous carcinoma are found in but two sections; however, they are so striking and picturesque as to defy misinterpretation.

The neoplastic process of the gastric mucosa stops abruptly at the junction of pylorus with duodenum, although the submucosa and outer layers of the duodenum are heavily infiltrated by intermingled adenocarcinoma and epidermoid carcinoma.

The two small lymph nodes dissected out from the surgical speci-

men show the sinuses crowded with typical and disorderly mucous glands (adenocarcinoma).

The autopsy material shows that the esophagus and cardia were entirely normal. Below this level, but outside of the area of leathery induration, a low grade chronic productive inflammatory process prevails. This changes abruptly into a diffusely infiltrating adenocarcinoma at the margin where the gastric mucosal pattern becomes obliterated. The indurated mass proper represents a compact, diffusely infiltrating adenocarcinoma showing all the stigmata of a rapidly proliferating carcinoma. The subserosal infiltrations formed mucin in abundance and tumor thrombi in this zone are very common.

While the involved portion of the stomach remaining after partial gastrectomy shows predominantly adenocarcinoma, an occasional focus of epidermoid carcinoma occurs in the line of the old scar, appearing independent of the glandular carcinoma.

The omentum, the lymph nodes and a small piece of pancreas are infiltrated only by adenocarcinoma. The adrenal gland is involved in scar tissue but not by tumor.

DISCUSSION

Squamous cell carcinoma at the gastro-esophageal junction and in the adjacent stomach is not particularly uncommon, but primary squamous cell carcinoma of the cardia is infrequent and difficult to establish as primary there. Squamous cell carcinomas of the stomach usually represent metastases, extensions or implantations from the tongue or from the esophagus. In these cases there is frequently a history of difficulty in swallowing of short duration, rapidly proceeding to esophageal obstruction.

Occasionally the body of the stomach is so extensively involved that the infiltration of the cardiac orifice entirely escapes notice and there is every appearance of a tumor arising in the stomach rather than in the esophagus or at the gastro-esophageal junction. Kaufmann's case, recorded by Herxheimer,⁴ presented a large cancerous growth of the posterior wall of the stomach, composed of cornifying squamous epithelium that extended to the esophageal junction. In this case a traumatic ulcer of the cardia was epithelialized from the esophagus and gave rise to the neoplasm. The tumor in Boyden's⁶

case was apparently in the stomach rather than in the esophagus, but it was so high up in the stomach that it completely surrounded the cardiac orifice and in one or two areas had grown along beneath the submucosa beyond the sphincter, so that there was a small amount of tumor actually above the cardia. The gross appearance suggested stomach but the histology was more in favor of esophageal origin. The case reported by Vinson and Broders⁷ falls into the above category.

At this point attention is invited to a finding which was reported by Prof. C. Toldt⁸ as long ago as 1880, and which has apparently been overlooked by present-day writers, although it is mentioned by von Kölliker.⁹ In some children between the ages of 1 and 4 years he found islands of stratified pavement epithelium between the cardiac glands of the stomach beyond the lower end of the esophagus. This has been rediscovered any number of times and new interpretations attached to it. Weidman¹⁰ reported a typical case in a 5 day old infant dying of acute gastritis, under the title of "Heteroplastic Esophageal Mucosa in Stomach." He, too, remarks that these islets of epithelium are not at all uncommon in the stomach. Of course the normal occurrence of islets of stratified squamous epithelium in the gastric cardia can be invoked as a source of squamous cell carcinoma in that location. But no such islets have been observed in the pylorus.

It is indeed coincidental that the case now reported combined all the features noted by Herxheimer,⁴ and by Oberling and Wolf,⁵ and in addition the major tumor was predominantly an epidermoid carcinoma, whereas in the 3 previously reported cases the epidermoid carcinoma was only a minor portion of the tumor or an accidental finding.

Histogenetic Remarks: Heterotopic squamous epithelium has never been observed in the pylorus. There is no basis for assuming its presence there. The histological picture clearly shows all stages in the transformation of cylindrical epithelium to the squamous type. Herxheimer made the same observation in the tumor reported by him. He, however, was reluctant to ascribe this change to metaplasia alone. He assumes that the change of the cell type does not take place until neoplastic cell growth begins, and then only in cells that are embryologically predisposed thereto.

Various hypotheses have been advanced to explain metaplasia

in such tumors. It would be only speculation to assume a presumptive cause in this case, whether it be inflammatory, regenerative or neoplastic.

NOTE: I wish to express my indebtedness to Drs. W. C. Teufel, G. C. Lake and J. F. Van Ackeren of the United States Public Health Service for the privilege of studying and reporting this case, and to Major V. H. Cornell, Curator of the Army Medical Museum, for the photographs.

SUMMARY

A case of cornifying epidermoid carcinoma occurring with an adenocarcinoma of the pylorus in which definite transitions from glandular to epidermoid carcinoma were present is reported.

The tumor removed at operation was predominantly epidermoid in character, confined to the pylorus and no metastases or other tumor foci were demonstrable.

At autopsy the esophagus and cardia were normal, the tumor in the vicinity of the gastric resection was predominantly adenocarcinomatous and the omentum, lymph nodes and pancreas were infiltrated only by adenocarcinoma.

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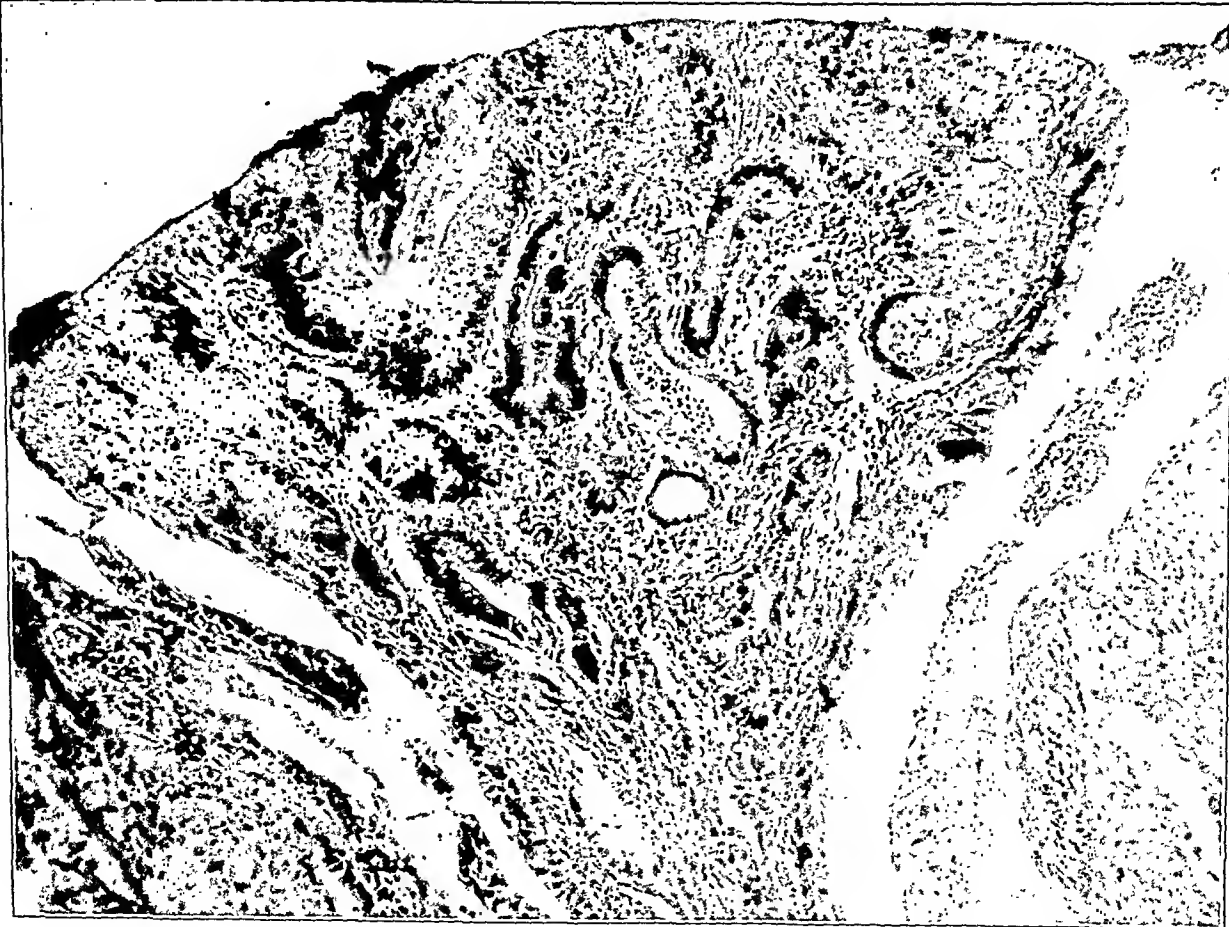
DESCRIPTION OF PLATES

PLATE 70

- FIG. 1. Thickened fold of pyloric mucosa showing abrupt change to epidermoid carcinoma. (Army Medical Museum Acc. 44510.) $\times 10$.
- FIG. 2. Transition of glandular to squamous cell carcinoma. (Army Medical Museum Acc. 44510.) $\times 200$.



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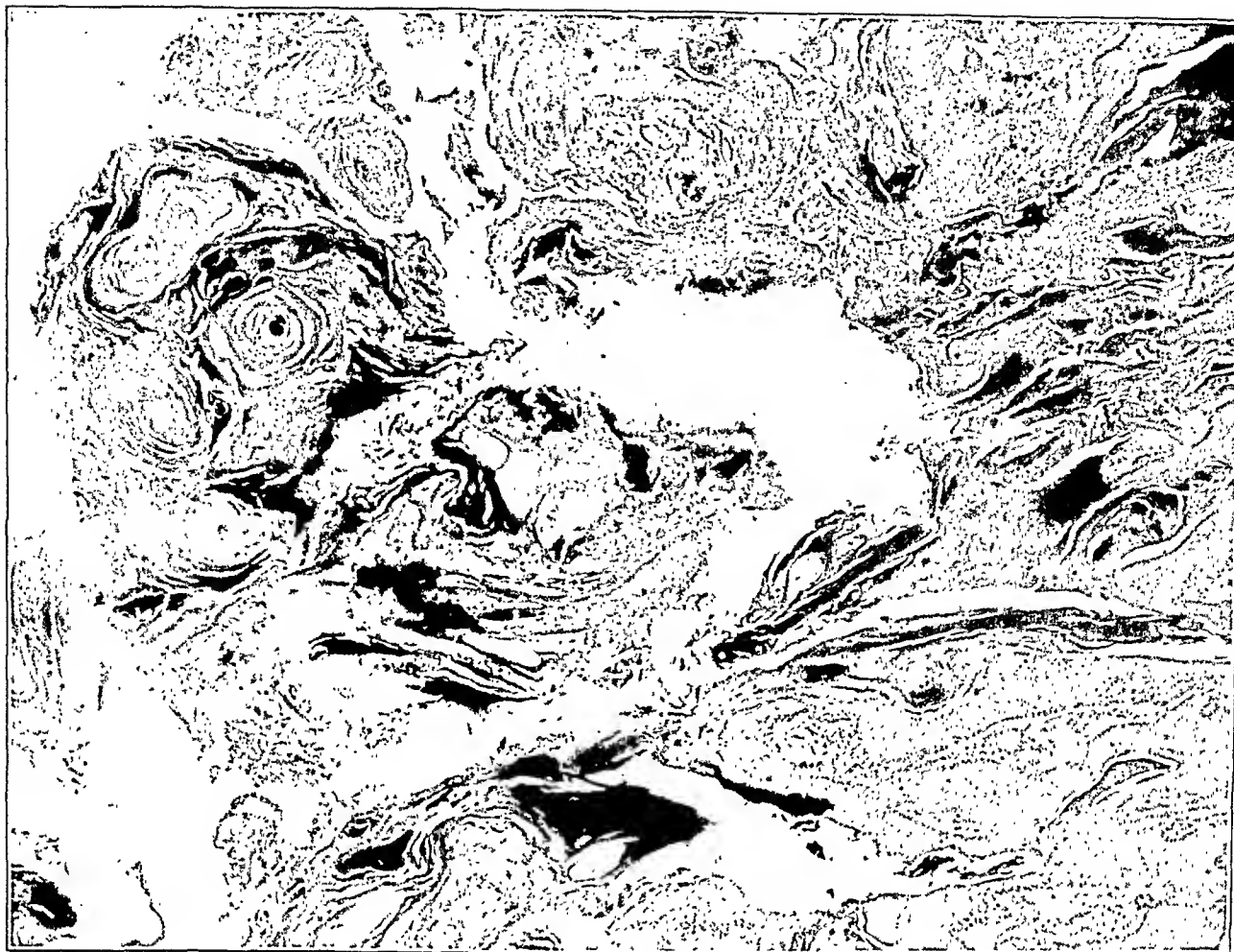


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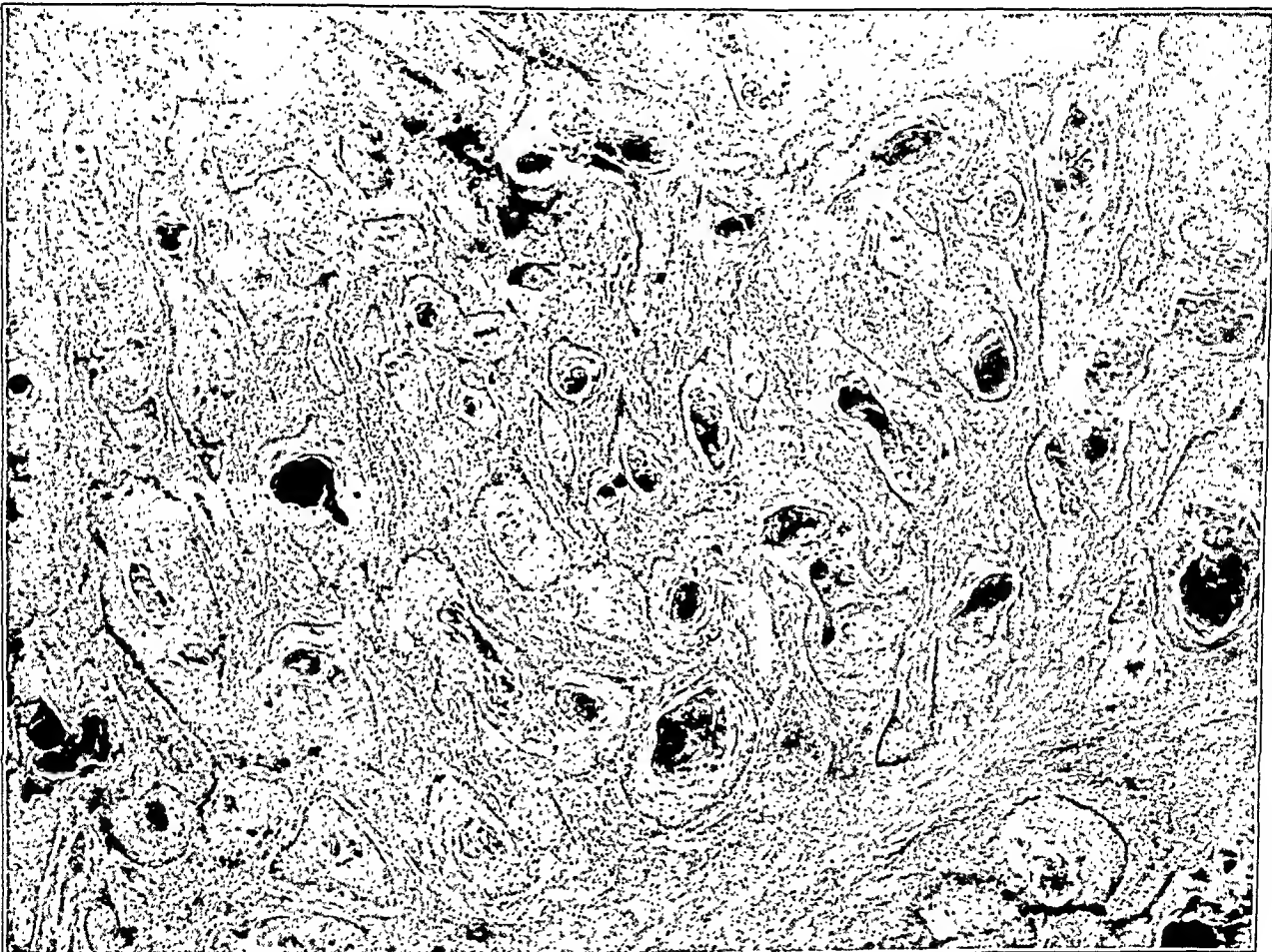
PLATE 71

FIG. 3. Cornifying surface of epidermoid carcinoma projecting into the lumen of the pylorus. (Army Medical Museum Acc. 44510.) $\times 60$.

FIG. 4. Pearl body formation. (Army Medical Museum Acc. 44510.) $\times 60$.



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4

REPORT OF A CASE PRESENTING AN ERYTHROBLASTIC TUMOR
IN THE THORACIC CAVITY

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Erythroblastosis is a condition in which an early fetal type of blood formation is maintained until term. The disease is characterized by a marked and progressive anemia, a blood picture showing great increase in nucleated red cells, a tendency to bleed from various mucous surfaces or organs, and not infrequently by fetal hydrops or icterus gravis neonatorum. Kleinschmidt ¹ believes the stage of hematopoiesis in these infants corresponds to that of the fetus of 3 to 5 months. That this name is descriptive only of one of a group of closely related conditions is obvious from a review of the literature. When both the erythroblastic and myeloblastic series are represented in the blood smears and histological preparations of the tissues it has been called erythroleukoblastosis, and when only the granulocytic series is present the term fetal leukemia has been used. There is more than conjecture to indicate that these conditions are different manifestations of an identical disease of the fetus.

In numerous instances these conditions have been found in association with two other pathological conditions of the fetus, congenital hydrops and icterus gravis neonatorum. Erythroblastosis has been seen without icterus gravis or hydrops but I have not been able to find a report of a case of congenital hydrops or icterus gravis without erythroblastosis, except those in which the examination of the fetus was not sufficiently complete to rule out its presence. One is led to assume that congenital hydrops and icterus gravis are conditions that frequently develop as a result of the blood dyscrasias known as erythroblastosis, erythroleukoblastosis and fetal leukemia.

As an example of this association I refer to a recent article by Ferguson ² reporting 6 cases of erythroblastosis, all of which were care-

* Read before the Omaha-Douglas County Medical Society, Omaha, Nebraska, May 29, 1934.

Received for publication November 20, 1934.

fully studied. Three of the 6 showed icterus, 2 marked generalized edema, and 1 neither jaundice nor edema.

The clinical features of the disease are as follows. The fetus dies shortly before birth, during birth, or the newborn infant lives but a short time; icterus, generalized edema, and bleeding from some mucous membrane, into the spinal cord, into the meninges, or from the kidneys have been most often observed; those that have hydrops are usually born dead or survive but a very short time. Anemia is marked and progressive; the nucleated cells may exceed 100,000 per cmm., the majority of them being immature erythroblasts or leukoblasts (Wanstrom's case showed 123,700, of which 49 per cent were nucleated red cells). If the infant survives for a time it is likely to have repeated abrupt attacks of cyanosis, in the last of which it dies.

The gross findings at autopsy may be listed as follows. There may be jaundice, congenital hydrops or marked anemia, enlargement of the liver and spleen, hypertrophy of the heart, and hemorrhages, particularly into the meninges. White nodules have been noted in the kidney and Plaut and Bullard³ observed abnormal smallness of the thymus. The placenta and cord show marked enlargement and increase in weight. Those having congenital hydrops show, in addition to the above, generalized edema, fluid in all the cavities, particularly ascites, and omental "cysts." The cases of icterus gravis have bile-staining of the brain and are likely to be covered at birth by a golden yellow vernix caseosa.

Histologically there is extensive embryonic hematopoiesis in the liver, spleen, pancreas, thymus, lymph nodes, adrenals, kidney, thyroid, and so on. No organ or tissue in which mesodermal tissues exist seems to be exempt. The bone marrow always shows active hematopoiesis. "Bile casts" in the liver are frequently mentioned by Ferguson² as typical findings and lipoidosis of the reticulo-endothelial cells of the liver is noted by Wanstrom.⁴

Discussion of the etiology of this group of apparently related conditions is out of place in this paper and at best could only restate a number of hypotheses. It may be permissible, however, to call attention to certain physiological facts concerning the fetus and to certain analogies with adult conditions.

A polycythemia in the newborn is practically a constant normal finding and is presumed to be a response to an oxygen-poor condition in fetal life. Goldbloom and Gottlieb⁵ have shown that every new-

born infant has in its blood a large number of immature red cells, either nucleated or reticulated. A reduction in number of red blood cells takes place rapidly the first 24 hours after birth and is down to normal at about 1 week. Coincident with this the immature red cells disappear from the blood stream. During the time they are disappearing an increase in bilirubin takes place in the plasma, leading to visible or concealed jaundice — *icterus neonatorum*.

These authors show further that if the blood cells of the newborn infant are kept in a test-tube, either in contact with the infant's plasma or with physiological sodium chloride solution, an analogous hemolysis takes place and the cells which vanish are, likewise, the immature forms.

There is then, apparently, a fetal mechanism designed for a definite physiological purpose, which bears certain resemblances to erythroblastosis and which, if exaggerated or maintained until term, may become an erythroblastosis.

Extramedullary resumption of blood formation has been seen to occur in several conditions. In a number of instances ^{6, 7} blood-forming tumors in adult life have been described, usually located in the retroperitoneal tissues.

In 1933 Jaffé ⁸ pointed out the probable relation of polycythemia vera to the leukemias. One must assume from his work that the excessive production of red cells in erythremia is analogous to that of the white cells in leukemia, especially of the myelocytic type. Furthermore, he offers proof that all cases of leukemia show unusual erythropoiesis in the bone marrow.

We have, of course, seen the so-called leukemic infiltrations in various organs in the course of this disease and accept the present interpretation that these "infiltrations" are areas in which blood formation from primitive mesenchymal tissue, analogous to that seen in the fetus, has been resumed.

It seems to me that the analogy between erythremia and leukemia in the adult and the erythroblastosis and leukoblastosis in the fetus is highly suggestive that the fetal conditions indicated by the various terms erythroblastosis, erythroleukoblastosis and fetal leukemia are identical and are fetal expressions of erythremia and leukemia. Possibly the terms fetal leukemia and fetal erythremia should be used and we should qualify the suitable term with such phrases as, "with hydrops fetalis," "with *icterus gravis neonatorum*," and so on.

The following report is of an unusual case in that in addition to the evident erythroblastosis there was a blood-forming tumor present in the left thoracic cavity of a newborn infant. I have not found such a condition reported.

REPORT OF CASE

Clinical History: Baby boy V., hospital No. 8357, Bryan Memorial Hospital. This baby was the third child of healthy parents. The first two children are living and well. They had no unusual jaundice or edema at birth. The mother had no signs of toxemia and was delivered normally at full term. The only abnormality noted during delivery was an unusually large placenta and cord. These were not weighed.

When about 12 hours old the infant began to have attacks of dyspnea, each of which subsided in a short time. At 32 hours he suddenly became deeply cyanotic. The cyanosis soon disappeared and was replaced by marked pallor. The infant was brought to the hospital when 36 hours old on March 12, 1933.

Examination upon admission showed weight 7 pounds and temperature 101° F. The left half of the chest was smaller than the right, was dull on percussion and no breath sounds were audible over it. The baby held its legs flexed on the thighs and thighs flexed on the abdomen. He had continuous priapism. There was a little oozing of blood from the cord, which stopped on retying. A provisional diagnosis of pulmonary atelectasis was made.

The baby was placed in a Drinker respirator for 12 hours and seemed to improve. When taken out, high pitched breath sounds could be heard over the left lung, the temperature was normal and his color good.

The hips were X-rayed and found to be normal, though the unusual position of the lower extremities persisted and it was very difficult to pull the legs and thighs down to normal posture. Evidently spasticity was present. The priapism was not constantly present after the first few days in the hospital.

No blood examination was made but the urine was examined and found normal. No bleeding from any mucous membrane was observed.

On the fifth hospital day he developed edema of the extremities and face and again showed marked cyanosis. The edema lasted for about 2 days, during which he gained from 4 to 6 ounces per day. The edema and cyanosis then rapidly disappeared and on the 17th day he was taken home apparently in good condition.

The day after dismissal the baby again became blue, the abdomen greatly distended and the rectal temperature rose to 104° F. He was readmitted and placed in the respirator. There was increasing cyanosis and he died at 6 P.M. on the day of the second admission.

ABSTRACT OF AUTOPSY PROTOCOL

The most striking abnormality was a tumor 4.5 by 6 cm. in diameter and 2 cm. thick, firmly attached to the posterior wall of the left pleural cavity and intimately connected with the hilum of the lung, so that the left main bronchial stem and the pulmonary artery

passed through a small portion of the tumor. In removing this tumor from the chest wall it was torn and several cc. of a semifluid material somewhat resembling pus escaped.

On examination the tumor (Fig. 6) had an irregular disc shape and was covered on the visceral surface by a smooth thin membrane resembling pleura. The color of this surface was reddish blue, resembling that of spleen. The surface attached to the chest wall and that portion connected with the hilum were torn, irregular and ragged. There was a central necrotic area from which the pus-like material had escaped. The tissue surrounding this cavity and composing the greater part of the mass was of unusual consistence and mottled in color from gray through the reds to brown.

The left lung was considerably smaller than the right and composed of two lobes. The lung was air-containing throughout but dark red and moist on the cut surface. There were a number of small, sub-pleural hemorrhages.

The organs were not weighed. The spleen and liver were recorded as about normal in size. The liver was rather meaty and deep red. The spleen was dark red on the cut surface.

Other significant abnormalities noted were a rather marked edema of the omentum so that it seemed distended with fluid, and an enlargement of the testicles, apparently due to edema.

An examination of the central nervous system was not permitted. The spasticity of the legs and the priapism suggested a lesion of the cord or of the cerebrum, perhaps hemorrhage or infiltration, and it is to be regretted that we could not examine them at autopsy.

HISTOLOGICAL EXAMINATION

The material studied consisted of sections of heart, lung, spleen, liver, suprarenal gland, kidney and the thoracic tumor. Sections of these tissues were stained with hematoxylin and eosin. In addition, sections of the tumor were stained with Wright's stain, Castroviejo's modification of Van Gieson's stain, Bielschowsky, hematoxylin and eosin-azur II, and the azocarmine modification of Mallory's collagen stain. The unusual findings are limited to the liver, suprarenal gland, spleen and the tumor.

Liver: The normal liver structures are well formed and well preserved. Scattered throughout the section, but somewhat more pro-

fusely near the capsule, are irregular islands of foreign cells. These cells are in groups, having from a very few to many cells per group. A nest of cells may lie isolated or many groups may be seen in a limited area (Fig. 1). They lie in the sinusoids, which are distended by them.

The cells themselves (Fig. 3) are much smaller than the liver cells, roughly spherical, though when crowded they may be somewhat fusiform. The nuclei are hyperchromatic with a distinct nuclear membrane. They usually show a single nucleolus. The chromatin is scattered in masses and thread-like pieces. The cytoplasm is relatively narrow, has a faintly granular appearance and is somewhat basophilic. When these cells are crowded together closely they give the impression of being a syncytial mass but, at the periphery, cell outlines can be distinguished.

While the type of cell just described predominates, one can find all stages of erythroblast formation. One may observe gradual assumption of hemoglobin with resulting polychromatophilia, gradual shrinking and condensation of the nucleus, mitotic figures, and finally mature erythrocytes.

Much less easily found in the liver are the various stages of granulocyte development, but it is possible to identify myeloblasts and various types of myelocytes. No megakaryocytes are seen.

It seems certain that the islands of young cells are hematopoietic in character and essentially erythropoietic. The young cells correspond in every way to those described and illustrated by Maximow⁹ as proerythroblasts and by Jaffé⁸ as erythrogonia.

Suprarenal Gland: In the suprarenal gland, in the inner border of the zona reticularis, a few similar hematopoietic foci are found (Fig. 2).

Spleen: Erythroblastic activity is present in the spleen and the whole series from the proerythroblast to the erythrocyte can be traced. There is not, however, such distinct formation of hematopoietic islands as seen in the liver and suprarenal gland, and it seems that here the process is less evident than in any of the other organs described.

Tumor: The tumor is surrounded by a capsule of loose connective tissue and covered on the free surface by a mesoendothelium. From the capsule numerous septa of loose connective tissue project into the mass at approximately right angles. These septa split and re-

unite in such a way as to divide the tumor into numerous alveolus-like spaces. These spaces vary greatly in size and are filled with cells (Fig. 4), the character of which will be described and illustrated below. There is no reticulum within the alveolus-like spaces and the few definite blood vessels present are in the septa and capsule.

Attention is first directed to the septa. They are composed of loose connective tissue, the ground work of which is homogeneous, contains but few fibrils and in many instances none. In this ground substance are seen nuclei varying from elongated, bluntly fusiform to roughly spherical in shape. These nuclei are rather vesicular and have a sparse, loosely arranged chromatin network.

At the ends of these septa, along their margins and within them, certain changes are seen to take place, these changes being identical wherever they occur. They are more easily traced when they occur within the septum and are as follows. The nuclei, described above as elongated and bluntly fusiform with loose chromatin arrangement, become rounded and the chromatin more dense. Whenever this happens there is an accumulation of several nuclei, the ground substance liquefies and a space is formed in which the cells containing these nuclei are seen to lie (Fig. 5). At this stage these nuclei resemble those described in the liver as erythrogonia or proerythroblasts (compare Figs. 3 and 5) and are surrounded by a narrow field of basophilic cytoplasm like those in the liver. The space in which they lie is not lined by endothelium.

As a result of these changes the margins of the septa are lined with, and the spaces within them are filled with, cells similar to the proerythroblasts of the liver and they develop from the cells of the primitive mesenchyme-like tissue of the septa. As one leaves the septa and examines the cells within the alveolus-like space between them all stages of development of the erythrocyte are seen. The mature red cells are as a rule most numerous near the central part of the alveolus.

Myeloblasts, myelocytes and mature granulocytes are less numerous than in the liver, though occasionally a small area can be found in which they are present. One cell closely resembling a megakaryocyte was found.

It is my belief that the process described in the liver, in the spleen, in the suprarenal gland and in the tumor is almost pure erythroblastic activity, and that the tumor is an erythroblastoma.

Unfortunately, bone marrow, pancreas, thymus and central nervous system were not represented among the tissues preserved at autopsy.

SUMMARY

A brief review of the pertinent literature is given, with special reference to the relation between erythroblastosis, erythroleukoblastosis and fetal leukemia.

Attention is drawn to the fetal mechanism apparently designed to meet oxygen-poor conditions of intrauterine life, an erythremia with a large number of immature red blood cells, and to their rapid reduction in number immediately following birth.

The possible analogy is pointed out between erythroblastosis and fetal leukemia on the one hand, and adult polycythemia vera and leukemia on the other.

The probable rôle of fetal hydrops and icterus gravis neonatorum as complications of erythroblastosis and fetal leukemia is stressed.

Finally, a case of erythroblastosis in a newborn infant having an erythroblastoma in the left pleural cavity is reported with autopsy findings and description of the histopathology.

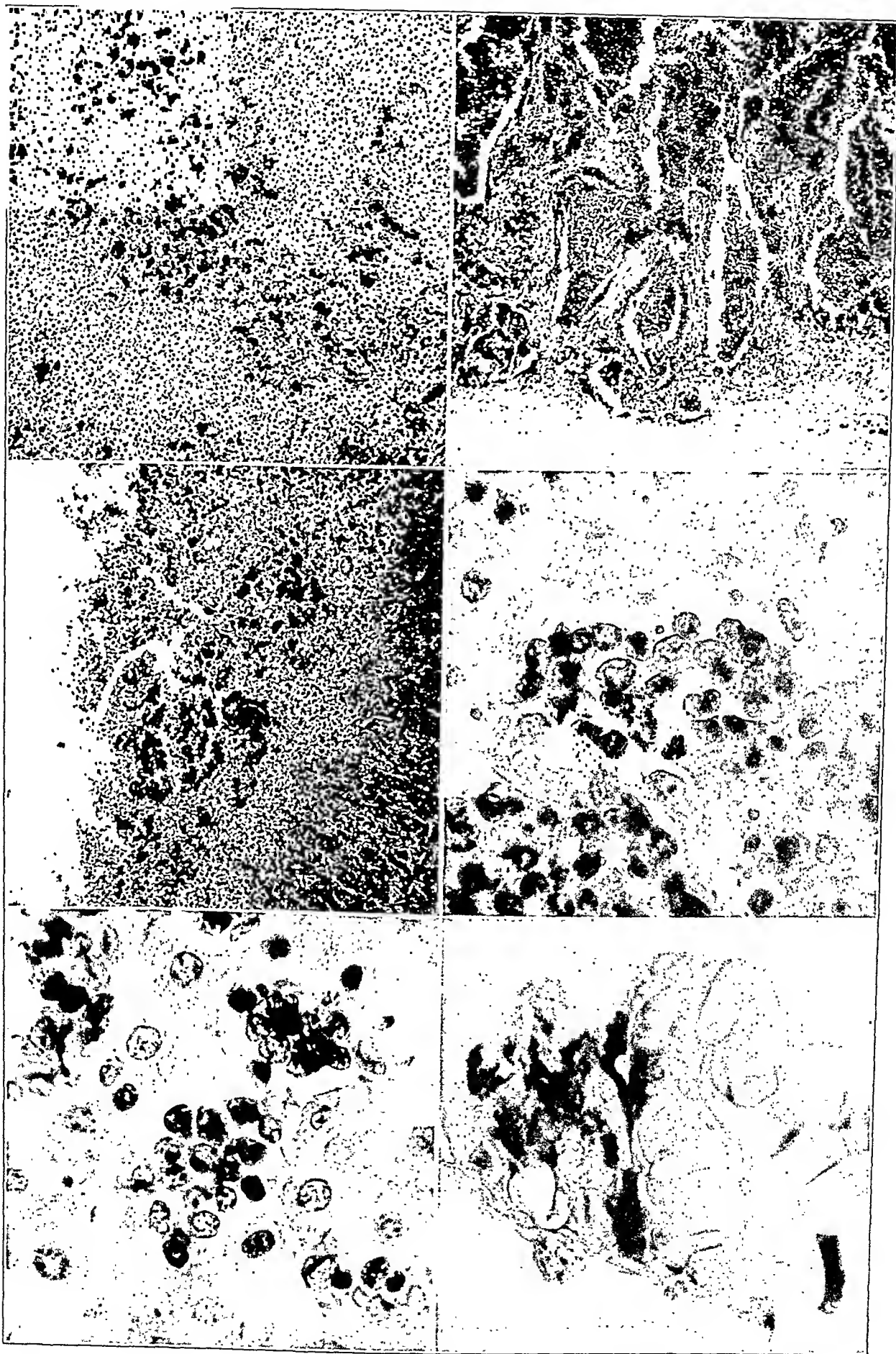
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DESCRIPTION OF PLATE

PLATE 72

- FIG. 1. Liver. Islands of embryonic blood cells lying in the sinusoids of the liver are seen as dark masses of varying size and irregular distribution.
- FIG. 2. Suprarenal gland. Islands of embryonic blood cells are seen in the zona reticularis.
- FIG. 3. Liver. One of the collections of embryonic blood cells occupies the central portion of the illustration. Most of these cells are proerythroblasts, although several normoblasts are present.
- FIG. 4. Thoracic tumor. Note the septa dividing the tumor into irregular, alveolus-like spaces which are filled with cells.
- FIG. 5. Thoracic tumor. A nest of proerythroblasts in a space within one of the septa. Note the likeness to the cells in Fig. 3. A few normoblasts and intermediate forms may be seen here also.
- FIG. 6. Thoracic tumor. The irregular margin at the left is the portion which was attached to the hilum structures of the left lung.



Covey

Erythroblastosis

ALTERATION IN SERUM BILIRUBIN AND BROMSULPHALEIN
RETENTION IN RELATION TO MORPHOLOGICAL CHANGES
IN THE LIVER AND BILE PASSAGES IN CATS WITH
TOTAL BILIARY STASIS *

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Experimental obstruction of the common bile duct has been employed by a number of investigators for the purpose of studying various problems of hepatic and biliary tract function and the pathogenesis and pathological basis of obstructive jaundice. There have been comparatively few attempts, however, to correlate changes in hepatic function with the morphological changes in the liver at varying intervals during total bile stasis in experimental animals.

The present study consists of determinations of the serum bilirubin concentration and degree of bromsulphalein retention in a series of 29 cats with uncomplicated total bile stasis produced by ligation of the common duct. Sections of liver were secured practically simultaneously with the chemical observations in most cases, either at laparotomy or autopsy. Simultaneous morphological and functional data were thus obtained for almost every 24 hour period up to the 16th day following ligation of the common duct. The morphological observations were supplemented by those made on a series of 46 cats previously described by Stewart and Lieber.¹

The animals employed were adult cats, maintained on a diet of fresh scrap meat and milk. Operation was performed under light ether anesthesia, the abdomen being carefully prepared by clipping, Harrington's solution and alcohol. The common duct was isolated, and in some cases was divided between ligatures, and in others was firmly ligated with $\frac{1}{8}$ inch tape close to the duodenum for the purpose of subsequent release of obstruction. The gall-bladder was left *in situ* in 10 cases, the remainder being subjected to either cholecystectomy (14) or cystic duct ligation (1). In 4 additional cases

* Received for publication November 21, 1934.

cholecystectomy or cystic duct ligation had been performed several weeks prior to ligation of the common duct. In several instances sections of normal liver were taken for purposes of comparison. In animals in which serial determinations of bilirubin and bromsulphalein retention were made, blood was obtained from the femoral vein. In the others, blood was withdrawn by cardiac puncture. Sections of liver were obtained either at laparotomy or autopsy. All cases that showed evidence of infection (other than a small localized stitch abscess) were excluded, as were several in which spontaneous or traumatic perforation of the common duct had occurred. In no case was there any evidence of reconstruction of the common duct or of passage of bile into the duodenum through anomalous ducts. The animals were killed by incising the heart under light ether anesthesia. A few animals which died spontaneously were discarded if post-mortem changes were noted.

Pieces of liver were fixed in 10 per cent formalin. A portion of the fixed tissue was frozen and sectioned and stained for fat; the remainder was blocked in paraffin, cut and stained with hematoxylin and eosin. Serum bilirubin was determined by the method of van den Bergh, as modified by Thannhauser and Andersen.² Bromsulphalein was injected intravenously in the dosage of 2 mg. per kilogram of body weight, the degree of retention being estimated in specimens of blood withdrawn 30 minutes after administration of the dye.

RÉSUMÉ OF LESIONS ASSOCIATED WITH COMPLETE BILIARY STASIS IN THE BILE DUCTS OF CATS

The bile ducts undergo progressive but irregular dilatation, roughly in proportion to their original size. The walls of the extra-hepatic ducts become attenuated and later slightly thickened. White bile may form rather rapidly. Proliferation of the smaller ducts is noted early, while the mucosa of the larger ducts undergoes enormous proliferation, particularly after the 10th day. By the 21st day the majority of the hepatic lobules are surrounded by a collar of proliferating small bile ducts. Destruction of these newly formed ducts may occur either as a result of distention with hyaline material and phagocytic cells or, in the late stages of stasis, as a result of the organization of areas of hyaline necrosis.

Pigmentation: Bile pigmentation is similar to that observed in

other animals but, in general, tends to be less marked and is not always proportional to the duration of stasis or the degree of bilirubinemia.

Regressive Changes: These are present (1) in the inner portion of the lobule, (2) immediately beneath the capsule of the liver, (3) sporadically throughout the hepatic parenchyma and (4) about the larger bile ducts. The necrotic cells in most situations are being constantly replaced by regenerated hepatic cells until the terminal stages of stasis, when the process of regeneration fails. In the subcapsular zone, however, the degenerative changes tend to remain stationary unless complete necrosis supervenes.

Focal Necrosis: Focal midzonal areas of necrosis are occasionally noted at the end of 5 hours, are most numerous between 24 and 48 hours and then decrease steadily, being entirely absent after the 13th day. The material in the areas of necrosis is cleared away and the parenchyma is restored to normal by regeneration of hepatic cells and by the ingrowth of liver cords from the surrounding tissue. Organization occasionally occurs if there has been a preliminary exudation of fibrin in the necrotic area.

Hyaline Necrosis: Within the first 24 hours the hepatic cells sporadically throughout the lobules, but especially immediately beneath the capsule, undergo a hyaline type of necrosis. The necrotic process occurring sporadically throughout the lobules is accompanied by a simultaneously developing regenerative process, as a result of which the necrotic cells are being constantly replaced by newly formed hepatic cells. The subcapsular areas of hyaline necrosis are frequently overlaid by a fibrinohemorrhagic peritoneal exudate. These areas undergo a characteristic type of repair which differs in several essential respects from that mentioned above. After the 5th day, increasing in frequency with prolonged stasis, an acidophilic hyaline type of necrosis appears which involves sporadically groups of 6 to 8 cells and variable portions of single or several lobules in the vicinity of portal radicles. These lesions undergo organization and the newly formed connective tissue, slowly contracting, pinches off and destroys the vascular and biliary channels of the neighboring portal radicles. The simultaneous progression of these processes of necrosis and fibrosis results in an extreme degree of concentric avascular fibrosis. Small collections of bile pigment are frequently seen in or about these fibrotic areas.

Vascular Changes: In the early stages there is usually dilatation of the branches of the hepatic and portal veins and of the lymphatic vessels in the portal radicles. The sinusoids are usually compressed by dilating bile ducts at the periphery of the lobules and may show focal or diffuse hyperemia in other areas. The blood vessels may be partially or completely occluded by hyaline or fibrinous thrombi, which may or may not be associated with focal midzonal areas of necrosis. There is a constant increase in the reticulum and connective tissue about all the vascular structures, particularly in the portal radicles where obliteration eventually occurs as a result of the organization of the hyaline necroses.

Regeneration: Evidences of regeneration are noted within 8 hours. These are characterized by budding and fission of the nuclei, leading to binucleation and multinucleation and to the formation of large hepatic cells with hyperchromatic nuclei. By the end of 48 hours, in the majority of cases, this type of regeneration gives way to the mitotic division of hepatic cells which predominates from this time on, although both processes may contribute to the maintenance of the normal hepatic parenchyma which is subject to repeated injury throughout the entire course of stasis. Regeneration lags after the 15th day, as evidenced by the more frequent occurrence of regressive lesions, which tend to progress, mitotic figures and binuclear cells appearing only occasionally.

EXPERIMENTAL DATA

Since practically no difference was noted in the morphological changes in the cholecystectomized and non-cholecystectomized animals, these two groups will not be separated in the presentation of the pathological lesions. In order to avoid unnecessary repetition, the findings characteristic of each day of stasis in the 29 animals included in the present study will be outlined and any unusual observations will be mentioned in the individual protocols.

One Day Stasis: Moderate dilatation of the extrahepatic ducts; absent to moderate dilatation of the medium sized intrahepatic ducts; absent to beginning proliferation of the smaller ducts; no thickening of the medium sized ducts; no proliferation of the mucosa of the bile ducts; subcapsular hyaline necrosis absent to marked; sporadic hyaline necrosis slight to moderate; sporadic nondescript

necrosis slight to marked; focal midzonal necroses absent to numerous; no fibrosis about portal radicles; slight to marked cytoplasmic vacuolization; no mitotic figures; pigmentation slight to moderate; occasional sinusoidal thrombosis.

CAT 130. 6/5/34. Ligation common duct, cholecystectomy. 6/6/34. Serum bilirubin 0.32 mg., bromsulphalein, no retention.

CAT 131. 6/6/34. Ligation common duct, cholecystectomy. 6/7/34. Serum bilirubin 0.68 mg., bromsulphalein, no retention.

CAT 132. 6/7/34. Ligation common and cystic ducts. 6/8/34. Serum bilirubin 2.04 mg., bromsulphalein 20 per cent.

Two Day Stasis: Moderate dilatation of extrahepatic ducts; slight to moderate dilatation medium sized intrahepatic ducts; slight proliferation smaller ducts; absent to slight thickening of medium sized ducts; slight to moderate proliferation of mucosa of ducts; subcapsular hyaline necrosis absent to stage of repair; sporadic hyaline necrosis slight to moderate; sporadic nondescript necrosis slight to marked; focal midzonal necroses frequent; beginning fibrosis about portal radicles; slight cytoplasmic vacuolization; no mitotic figures; slight to marked pigmentation.

CAT 143. 6/27/34. Ligation common duct, gall-bladder *in situ*. 6/29/34. Serum bilirubin 0.74 mg., bromsulphalein 35 per cent. Bile in ducts pale green.

CAT 144. 6/27/34. Ligation common duct, gall-bladder *in situ*. 6/29/34. Serum bilirubin 0.0 mg., bromsulphalein, no retention.

CAT 147. 7/3/34. Ligation common duct, cholecystectomy. 7/5/34. Serum bilirubin 2.4 mg., bromsulphalein 100 per cent.

Three Day Stasis: Moderate to marked dilatation extrahepatic ducts; slight dilatation medium sized intrahepatic ducts; slight proliferation smaller ducts; slight thickening medium sized ducts; slight proliferation duct mucosa; subcapsular hyaline necrosis present; sporadic hyaline necrosis marked; sporadic nondescript necrosis marked; focal midzonal necroses absent; slight fibrosis about portal radicles; moderate cytoplasmic vacuolization; no mitotic figures; pigmentation moderate.

CAT 83. 12/27/33. Ligation cystic duct. 3/12/34. Ligation common duct, cholecystectomy. 3/13/34. Serum bilirubin 0.84 mg., bromsulphalein 20 per cent. 3/14/34. Serum bilirubin 0.80 mg., bromsulphalein 45 per cent. 3/15/34. Serum bilirubin 0.76 mg., bromsulphalein 80 per cent.

CAT 88. 1/4/34. Ligation cystic duct. 3/5/34. Ligation common duct. 3/6/34. Serum bilirubin 0.35 mg., bromsulphalein 40 per cent. 3/7/34. Serum bilirubin 0.92 mg., bromsulphalein 40 per cent. 3/8/34. Serum bilirubin 1.4 mg., bromsulphalein 100 per cent.

Four Day Stasis: Moderate to marked dilatation extrahepatic ducts; moderate dilatation medium sized intrahepatic ducts; moderate thickening medium sized ducts; moderate proliferation duct mucosa; subcapsular hyaline necroses undergoing repair; sporadic hyaline necrosis marked; sporadic nondescript necrosis marked; focal midzonal necroses absent; beginning fibrosis about portal radicles; slight cytoplasmic vacuolization; no mitotic figures; slight pigmentation.

CAT 87. 1/4/34. Ligation cystic duct. 3/5/34. Ligation common duct. 3/6/34. Serum bilirubin 0.61 mg., bromsulphalein 100 per cent. 3/7/34. Serum bilirubin 1.12 mg., bromsulphalein 100 per cent. 3/9/34. Serum bilirubin 2.16 mg., bromsulphalein 100 per cent.

Six Day Stasis: Marked dilatation extrahepatic ducts; moderate to marked dilatation medium sized intrahepatic ducts; moderate to marked proliferation smaller ducts; moderate thickening medium sized ducts; moderate to marked proliferation duct mucosa; late stages of repair of subcapsular hyaline necrosis; sporadic hyaline necrosis slight to marked; sporadic nondescript necrosis slight to marked; focal midzonal necroses absent to slight; slight to moderate fibrosis about portal radicles; moderate cytoplasmic vacuolization; mitotic figures present; pigmentation slight to moderate.

CAT 145. 7/3/34. Ligation common duct, gall-bladder *in situ*. 7/9/34. Serum bilirubin 0.47 mg., bromsulphalein 5 per cent. Wide zone subcapsular degeneration.

CAT 146. 7/3/34. Ligation common duct, gall-bladder *in situ*. 7/9/34. Serum bilirubin 0.47 mg., bromsulphalein 5 per cent. Wide zone subcapsular degeneration and vacuolization.

CAT 63. 2/12/34. Cholecystectomy. 5/8/34. Ligation common duct. 5/14/34. Serum bilirubin 1.91 mg., bromsulphalein 70 per cent.

CAT 154. 7/10/34. Ligation common duct, cholecystectomy. 7/16/34. Serum bilirubin 15.2 mg., bromsulphalein 50 per cent.

Seven Day Stasis: Marked dilatation of extrahepatic ducts; moderate to marked dilatation of medium sized intrahepatic ducts; moderate to marked proliferation of smaller bile ducts; moderate to marked thickening of medium sized ducts; moderate to marked proliferation of duct mucosa; subcapsular hyaline necrosis absent; sporadic hyaline necrosis slight to marked; sporadic nondescript necrosis slight to marked; focal midzonal necroses absent; fibrosis about portal radicles slight to moderate; cytoplasmic vacuolization

moderate to marked; mitotic figures present; pigmentation slight to marked.

CAT 150. 7/3/34. Ligation common duct, gall-bladder *in situ*. 7/10/34. Serum bilirubin 4.5 mg., bromsulphalein 50 per cent. Smaller ducts disappearing on account of accumulation of hyaline material.

CAT 151. 7/3/34. Ligation common duct, gall-bladder *in situ*. 7/10/34. Serum bilirubin 8.5 mg., bromsulphalein 40 per cent.

CAT 114. 5/15/34. Ligation common duct, cholecystectomy. 5/22/34. Serum bilirubin 6.27 mg., bromsulphalein 100 per cent.

Eight Day Stasis: Marked distention of extrahepatic ducts; bile frequently pale green, thin consistence; slight to moderate dilatation medium sized ducts; marked proliferation smaller ducts; moderate to marked thickening medium sized ducts; moderate to marked proliferation duct mucosa; subcapsular hyaline necrosis absent; sporadic hyaline necrosis marked; sporadic nondescript necrosis marked; focal midzonal necroses absent or occasional; moderate fibrosis about portal radicles; cytoplasmic vacuolization slight; mitotic figures present; pigmentation moderate to marked.

CAT 82. 12/27/33. Ligation cystic duct. 3/12/34. Ligation common duct, gall-bladder atrophied and contracted. 3/13/34. Serum bilirubin 1.24 mg., bromsulphalein 30 per cent. 3/14/34. Serum bilirubin 1.72 mg., bromsulphalein 55 per cent. 3/15/34. Serum bilirubin 2.24 mg., bromsulphalein 80 per cent. 3/16/34. Serum bilirubin 3.16 mg., bromsulphalein 80 per cent. 3/19/34. Serum bilirubin 6.5 mg., bromsulphalein 30 per cent. 3/20/34. Serum bilirubin 6.8 mg., bromsulphalein 100 per cent.

CAT 120. 5/24/34. Ligation common duct, cholecystectomy. 6/1/34. Serum bilirubin 2.16 mg., bromsulphalein 50 per cent.

CAT 121. 5/24/34. Ligation common duct, cholecystectomy. 6/1/34. Serum bilirubin 3.18 mg., bromsulphalein 60 per cent. Numerous binucleate and hyperchromatic hepatic cells.

Ten Day Stasis: Marked to enormous dilatation extrahepatic ducts; bile commonly pale green or brown, variable consistence; moderate dilatation medium sized intrahepatic ducts; marked proliferation smaller ducts; moderate thickening medium sized ducts; marked proliferation duct mucosa; subcapsular hyaline necrosis occasionally present; sporadic hyaline necrosis marked; sporadic nondescript necrosis marked; focal midzonal necroses absent; fibrosis about portal radicles slight; cytoplasmic vacuolization slight; mitotic figures absent; pigmentation moderate.

CAT 77. 4/13/34. Ligation common duct, gall-bladder *in situ*. 4/14/34. Serum bilirubin 0.91 mg., bromsulphalein 45 per cent. 4/16/34. Serum bili-

rubin 1.31 mg., bromsulphalein 15 per cent. 4/18/34. Serum bilirubin 1.6 mg., bromsulphalein 40 per cent. 4/23/34. Serum bilirubin 0.96 mg., bromsulphalein 55 per cent.

CAT 155. 7/13/34. Ligation common duct, cholecystectomy. 7/23/24. Serum bilirubin 6.5 mg., bromsulphalein 40 per cent. Marked compression of hepatic parenchyma about larger bile ducts. Areas of hyaline necrosis spreading out from larger portal radicles.

Eleven Day Stasis: Marked distention extrahepatic ducts; bile frequently dark green or brown, variable consistence; medium sized intrahepatic ducts frequently compressed, occasionally dilated; marked proliferation smaller ducts; marked thickening medium sized ducts; slight proliferation duct mucosa; subcapsular hyaline necrosis occasionally present; sporadic hyaline necrosis marked; sporadic nondescript necrosis marked; focal midzonal necroses absent; moderate fibrosis about portal radicles; cytoplasmic vacuolization moderate; no mitotic figures; pigmentation marked.

CAT 64. 4/23/34. Ligation common duct, gall-bladder *in situ*. 5/4/34. Serum bilirubin 1.88 mg., bromsulphalein 20 per cent.

CAT 72. 4/23/34. Ligation common duct, gall-bladder *in situ*. 5/4/34. Serum bilirubin 2.48 mg., bromsulphalein 30 per cent.

CAT 123. 5/24/34. Ligation common duct, cholecystectomy. 6/4/34. Serum bilirubin 4.8 mg., bromsulphalein 85 per cent. Localized sinusoidal congestion.

CAT 122. 5/24/34. Ligation common duct, cholecystectomy. 6/4/34. Serum bilirubin 4 mg., bromsulphalein 75 per cent.

Twelve Day Stasis: Enormous distention extrahepatic ducts; bile frequently dark green; medium sized intrahepatic ducts usually compressed; moderate to marked proliferation smaller bile ducts; marked thickening medium sized ducts; slight proliferation duct mucosa; subcapsular hyaline necrosis occasionally present; sporadic hyaline necrosis moderate; sporadic nondescript necrosis moderate; focal midzonal necroses absent; marked fibrosis about portal radicles, bile ducts and sublobular veins; very slight cytoplasmic vacuolization; no mitotic figures in hepatic cells but some in bile duct epithelium; pigmentation marked.

CAT 116. 5/10/34. Ligation common duct; gall-bladder *in situ*. 5/22/34. Serum bilirubin 2.92 mg., bromsulphalein 100 per cent.

Fifteen Day Stasis: Enormous dilatation extrahepatic ducts; compression of medium sized intrahepatic ducts; marked proliferation smaller ducts; marked thickening medium sized ducts; marked pro-

liferation duct mucosa with stretching and thinning of walls of larger intrahepatic and extrahepatic ducts; subcapsular hyaline necrosis absent; sporadic hyaline necrosis marked; sporadic nondescript necrosis marked; focal midzonal necroses absent; moderate fibrosis about portal radicles with obliteration of vessels and bile ducts; cytoplasmic vacuolization slight; no mitotic figures; pigmentation marked; marked compression and atrophy of parenchyma.

CAT 128. 5/28/34. Ligation common duct, cholecystectomy. 6/12/34. Serum bilirubin 1.44 mg., no bromsulphalein retention. Bile dark green.

Sixteen Day Stasis: Enormous distention extrahepatic ducts, bile at times dark green; compression medium sized intrahepatic ducts; marked proliferation smaller ducts; marked thickening walls medium sized ducts; proliferation larger duct mucosa with stretching and thinning of walls; subcapsular hyaline necrosis absent; sporadic nondescript necrosis marked; focal midzonal necroses absent; marked fibrosis about portal radicles with obliteration of vessels and ducts; cytoplasmic vacuolization slight; no mitotic figures; pigmentation slight to marked; large areas hyaline necrosis about portal radicles; marked compression and atrophy of parenchyma about larger ducts.

CAT 125. 5/26/34. Ligation common duct, cholecystectomy. 6/11/34. Serum bilirubin 3.4 mg., bromsulphalein 100 per cent.

CAT 129. 5/28/34. Ligation common duct, cholecystectomy. 6/13/34. Serum bilirubin 2.62 mg., bromsulphalein 30 per cent.

DISCUSSION

Serum Bilirubin

Bilirubin is not present in the blood of the normal cat in sufficient concentration to be detected by means of the van den Bergh reaction. As is true of the dog, from the standpoint of renal excretion it may be regarded as a non-threshold substance. In the present experiment no attempt was made to measure the urinary elimination of bile pigment, but it was obviously present in the urine in every case at the end of 24 hours of biliary stasis.

Several observers have studied the development of hyperbilirubinemia following common duct ligation. The time of appearance of a definite increase in serum bilirubin has varied from 5 minutes, as reported by Bollman, Sheard and Mann,³ to 3 hours, as noted by

Bloom,⁴ Snell, Greene and Rowntree⁵ and Barron and Bumstead.⁶ Snell, Greene and Rowntree⁵ found that in the dog only traces of bilirubin could be detected in the serum 24 hours after ligation of the common duct with the gall-bladder *in situ*; in 48-72 hours the serum contained 2-4 mg. of bilirubin per 100 cc. and bilirubin appeared in the urine. The degree of bilirubinemia increased progressively until the 2nd or 3rd week, remaining rather constant thereafter with slight daily fluctuations. In animals in which cholecystectomy was performed simultaneously with duct ligation, an increase in the serum bilirubin concentration was noted within 30 minutes, rising to 0.3 mg. at the end of 90 minutes, to 2.2 mg. in 4 hours and to 3-5 mg. at the end of 24 hours. It continued to increase steadily during the first weeks, when a transient decrease occurred, followed by a subsequent rise to a rather constant level. The highest concentration reported by Snell, Greene and Rowntree⁵ was 13.4 mg. per 100 cc., at the end of 17 days of stasis. Jordan and Greene⁷ observed an increase to 13.5 mg. at the end of 9 weeks of complete stasis and, in one instance reported by Salmon,⁸ the serum bilirubin rose to 30 mg. per 100 cc. on the 3rd day. The figures reported by the latter, however, are uniformly considerably higher than those noted by other observers.

The influence of the gall-bladder on the development of hyperbilirubinemia following obstruction of the common bile duct has been demonstrated by Mann and Bollman⁹ and Snell, Greene and Rowntree,⁵ who showed that jaundice increases more rapidly in dogs following cholecystectomy, ligation of the cystic duct or chemically produced cholecystitis. This is explained on the basis of the observation of Rous and McMaster¹⁰ that an intact gall-bladder possesses the ability to concentrate the bile to a marked degree, thereby delaying the development of an increase in intraductal pressure and hepatic injury following ligation of the common duct. Although marked individual variation in the degree of bilirubinemia was exhibited by the animals in the present study, those with gall-bladders did not differ essentially from those in which cholecystectomy or cystic duct ligation had been performed. In two instances, however (Cats 145 and 146), the serum bilirubin concentration was only 0.47 mg. on the 6th day of stasis; this figure is considerably lower than any noted at a similar stage in cholecystectomized animals. The direct van den Bergh reaction was positive in every case in which bilirubin could be detected in the blood.

The highest serum bilirubin concentration in this series was 15.2 mg. per 100 cc. (Cat 154), obtained on the 6th day of stasis. The marked individual variation in the degree of hyperbilirubinemia is apparent from the data present in Charts 1 and 2. No morphological basis for this variability could be observed. The bile duct changes

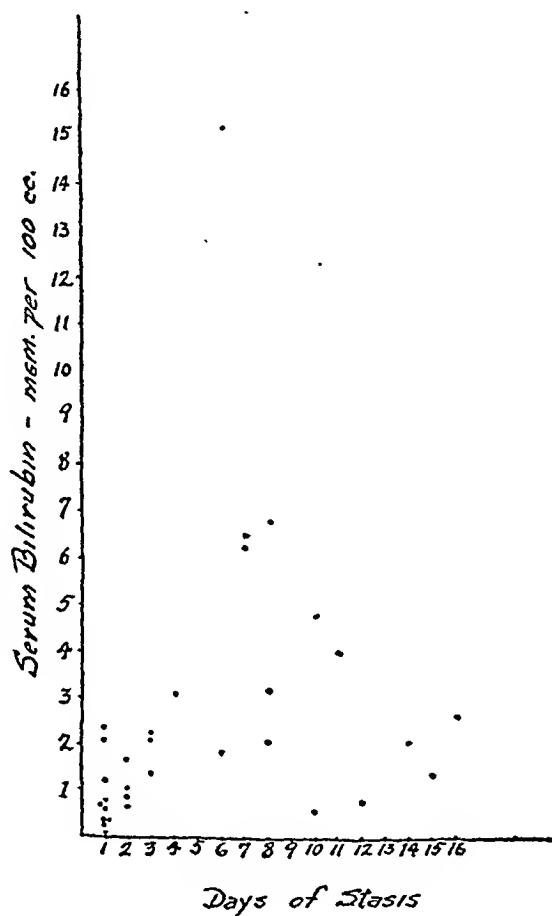


CHART 1

Distribution of serum bilirubin values during total biliary stasis in 15 cholecystectomized cats and 4 with cystic duct ligation.

appeared to progress consistently throughout the experimental period. Sporadic hyaline and nondescript necrosis of hepatic cells varied considerably and seemed to bear no relation to the individual variation in serum bilirubin concentration. The same was true of the focal midzonal areas of necrosis which increased to reach a maximum at the end of about 48 hours and then practically disappeared, being rarely seen in the later stages. Fibrosis about the portal radicles increased steadily with the duration of stasis and became rather promi-

ment at about the time when the serum bilirubin began to fall, but this relation was not constant. The degree of bilirubinemia was also apparently independent of the presence and extent of hepatic cell regeneration in individual cases. However, in the present series, the tendency toward a drop in serum bilirubin approximately coincided with the time of disappearance of mitotic figures from the hepatic cells (10th day). A similar lack of correlation existed with regard to

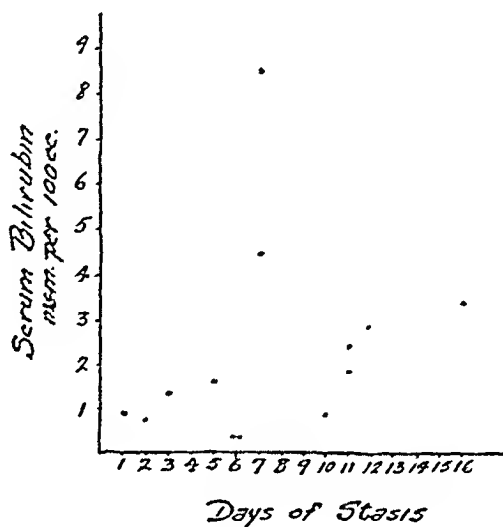


CHART 2

Distribution of serum bilirubin values during total biliary stasis in 10 cats with the gall-bladder *in situ*.

the lipid content of the hepatic and Kupffer cells, which was studied in detail and will be described in a subsequent communication. These observations seem to indicate that there is no demonstrable correlation, in individual instances, between the changes in the liver and bile ducts and the serum bilirubin concentration at any given time during the period of total bile stasis.

Analysis of the data indicates that, contrary to natural expectation, the serum bilirubin concentration does not tend to increase steadily during the period of stasis. Routine determinations were made at frequent intervals in only 4 animals (Chart 3). In general, the distribution of the serum bilirubin values, as presented in Charts 1 and 2, is in accord with the observation of other investigators that, following ligation of the common duct, the rather rapid initial rise is followed by a fall to a comparatively low level. Snell, Greene and

Rowntree⁵ stated that in non-cholecystectomized dogs the degree of bilirubinemia increased progressively until the 2nd or 3rd week and then remained constant, aside from slight daily fluctuations. In cholecystectomized animals the serum bilirubin rose steadily during the 1st week after ligation of the common duct, when a transient decrease occurred, followed in some instances by a secondary rise to the final level. Similar findings were reported by Salmon⁸ and by Jordan and Greene.⁷

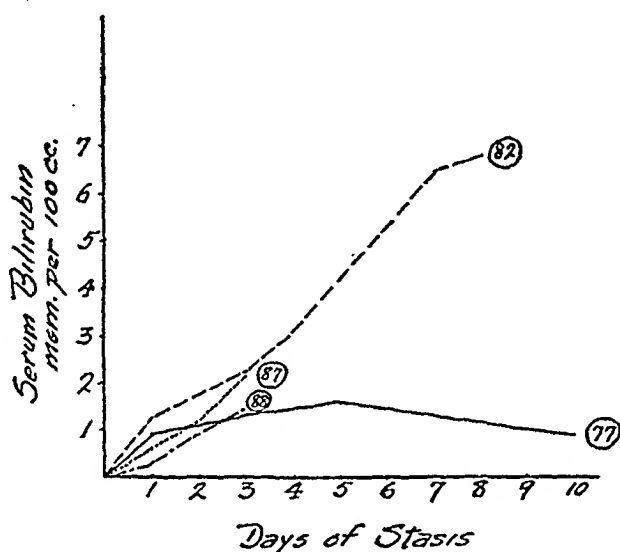


CHART 3

Serial determinations of serum bilirubin concentration during total biliary stasis in 4 cats.

Salmon⁸ suggests that this decrease in serum bilirubin concentration may be due to exhaustion of the bilirubin-forming mechanism, the reticulo-endothelial system, associated as it was in his cases with a profound, progressive asthenia. Jordan and Greene⁷ discuss in detail the relation between the degree of bilirubinemia and the metabolism of hemoglobin in experimental obstructive jaundice. It has been shown that ligation of the common bile duct of experimental animals is commonly followed by a gradual fall in hemoglobin and the work of Rous and Drury suggests that there is a definite relation between changes in the serum bilirubin concentration and variations in the percentage of hemoglobin. As a result of their studies of anemia in experimental obstructive jaundice, Jordan and Greene⁷ concluded that the degree of bilirubinemia seems to vary with the amount of blood being regenerated and with the activity of the me-

tabolism of pigment, rather than with the total amount or percentage of circulating hemoglobin. It appears likely that these factors may be largely responsible for the marked individual variation in the degree of obstructive hyperbilirubinemia noted in the present experiment, as well as in those reported by Snell, Greene and Rowntree,⁵ Salmon⁸ and Jordan and Greene⁷ in dogs, and by Cameron and Oakley¹¹ in rats. However, the phenomenon of the fall in serum bilirubin following the initial increase appears to be too constant to be dependent upon these exceedingly variable factors. Nor can it be readily explained on the basis of a suddenly increased rate of elimination of bile pigment in the urine for, as reported by Salmon,⁸ there is a close parallelism between the blood bilirubin concentration and the renal excretion of bilirubin in dogs with obstructive jaundice. Similarly, Brakefield and Schmidt¹² found that the maximum elimination of bile pigment occurs shortly after the onset of jaundice, the output subsequently declining during the period of continued stasis and reaching a low but somewhat constant level.

There is one factor that has received comparatively little consideration in this connection. Malkoff¹³ and Brakefield and Schmidt¹² have shown that when the common bile duct is ligated the bile acid content of the urine increases rapidly and then falls gradually to a comparatively low level. According to the latter, "the output begins to decline about a week after the onset of biliary stasis and finally reaches a value which in some instances is probably within the limits of accuracy of the method employed." The observations of Varela Fuentes, Apolo and Esculies,¹⁴ Snell, Greene and Rowntree¹⁵ and Rowntree, Greene and Aldrich¹⁶ indicate a corresponding change in the bile acid content of the blood. The fact that the time at which the diminution in bile acids occurs approximately coincides with the secondary drop in the serum bilirubin concentration may be of significance. This problem is at present under investigation.

Bromsulphalein Retention

In his original observations, Rosenthal¹⁷ found that abnormal retention of phenoltetrachlorophthalein did not occur during the first 3 days following ligation of the common bile duct. However, Snell, Greene and Rowntree,⁵ employing a dosage of 10 mg. per kilogram of body weight (double the clinical dosage), reported beginning

retention in from 48 to 72 hours in non-cholecystectomized dogs and within 24 hours in cholecystectomized dogs. They found that dye retention approximately paralleled the curve of bilirubinemia during the period of stasis. After intensive trial in a large number of cats we found that although bromsulphalein tends to disappear from the blood stream more rapidly than in human beings, nevertheless the

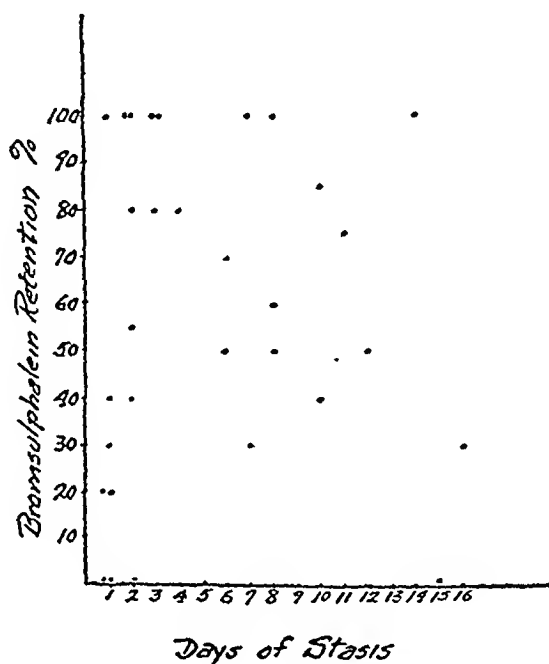


CHART 4

Distribution of bromsulphalein retention values during total biliary stasis in 15 cholecystectomized cats and 4 with cystic duct ligation.

dose employed clinically, 2 mg. per kilogram, was the maximum quantity which would consistently be completely removed from the blood within 30 minutes. Our findings coincide with those of Snell, Greene and Rowntree⁵ in so far as the time of appearance of dye retention is concerned. Analysis of our data, however, indicates that contrary to their findings there is no constant relation between the degree of dye retention and the serum bilirubin concentration. Serial determinations made in 5 cases (Chart 6) reveal the same discrepancy. This lack of correlation was most striking in the group of cholecystectomized animals, particularly in the early days of stasis, but it was also present in the non-cholecystectomized group.

If one keeps in mind the fact that much more significance must be attached to high than to low values in these animals, the occurrence

of 100 per cent dye retention on the 1st day of stasis in 1 case (Cat 87), on the 2nd day in 2 cases (Cats 87 and 147) and on the 3rd day in 2 cases (Cats 87 and 88) and of values ranging from 40 to 80 per cent in several others within the first 72 hours, appears noteworthy. The range of individual variation in the degree of dye retention was even more marked than in that of bilirubinemia and was not con-

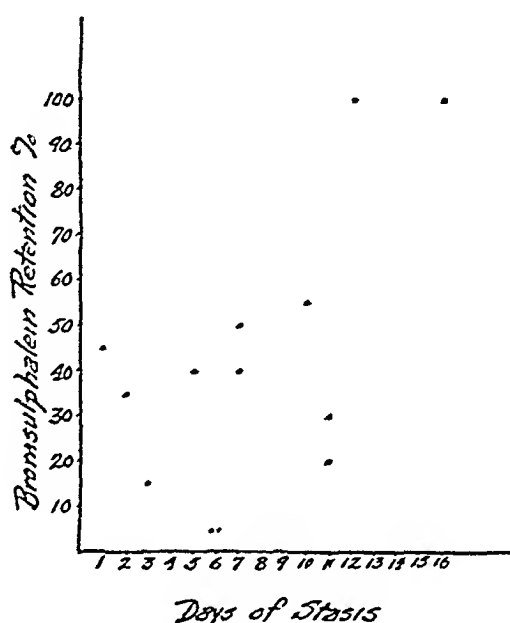


CHART 5

Distribution of bromsulphalein retention values during total biliary stasis in 10 cats with the gall-bladder *in situ*.

sistently related to the duration of stasis in the group as a whole, less so in the cholecystectomized than in the non-cholecystectomized animals (Charts 4 and 5). For example, 100 per cent retention was present in Cats 147, 88, 87, 114, 82, and 125 with stasis of 1 to 15 days duration, with serum bilirubin concentrations of 2.4, 1.4, 0.61, 6.27, 6.8 and 3.4 mg. respectively. As was the case with the serum bilirubin content, there was no apparent relation between the degree of bromsulphalein retention and the morphological changes in the liver and bile ducts, either in individual instances or in the group as a whole. It was noted, however, that the degree of dye retention appeared to parallel the clinical condition of the animal more closely than did any other factor included in the experimental observations. The most marked retention almost invariably occurred in cats that

were obviously quite toxic, although relatively low grades of retention were occasionally observed in such animals.

The immediate marked retention of bromsulphalein which occurred in some cases might conceivably have been due in part to the simultaneous removal of the gall-bladder, as was noted clinically following cholecystectomy by Cantarow, Gartman and Ricchiuti.¹⁸ However, in Cats 87 and 88, which showed 100 per cent retention 24 hours after ligation of the common duct, the cystic duct had been

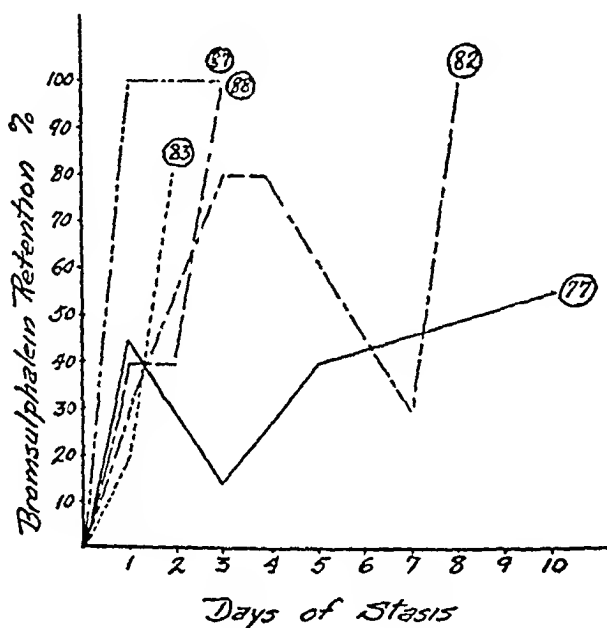


CHART 6

Serial determinations of bromsulphalein retention during total biliary stasis in 5 cats.

ligated 2 months previously. It seems evident that the degree of bromsulphalein retention exhibited by cats with complete common duct obstruction does not depend entirely upon the condition of the hepatic parenchyma or the bile ducts. Snell, Greene and Rowntree⁵ suggest the possibility of the accumulation of the dye in the obstructed biliary passages with subsequent reabsorption. However, no dye was found in the bile ducts of their animals, although they had received repeated doses during the period of stasis. On the other hand, it has been noted repeatedly that all dye leaves the blood stream within a few hours, regardless of the state of the liver.

These apparently contradictory observations may be explained on the basis of one of three hypotheses: (1) extrahepatic elimination,

(2) storage, or (3) destruction of the dye. It is well known that relatively small quantities of phenoltetrachlorophthalein and bromsulphalein are excreted by the kidneys of normal animals, but the quantity ordinarily eliminated in this manner is far too small (0.1-1 mg. in 2 hours) to account for the rapid removal of the dye from the blood. The question naturally arises as to whether much more might not be excreted in the urine in the presence of common duct obstruction. In this connection, Snell, Greene and Rowntree⁵ found that in general the amount of dye in 2 hour specimens of urine ran parallel to the degree of dye retention in the blood. Although it would appear that this hypothesis is untenable, it cannot be entirely eliminated from consideration on the basis of the limited amount of information now available.

The observations of Fiessinger and Longchamp¹⁹, Saxl and Donath²⁰ and Schellong and Eisler²¹ suggested that phenoltetrachlorophthalein is removed from the blood by cells of the reticulo-endothelial system and should be regarded as a test of the functional activity of this system rather than of the liver. A similar suggestion was made by Herlitz²² with regard to bromsulphalein. Klein and Levinson²³ found that splenectomy or reticulo-endothelial blockade with India ink resulted in definite delay in the rate of removal of this dye from the blood, this effect persisting for variable periods of time up to 30 days. They believe that bromsulphalein is excreted through the reticulo-endothelial system, the Kupffer cells playing an important rôle in this connection. Although the degree of dye retention reported by these observers was in most cases relatively slight, no statement can be made with regard to the possible alteration in reticulo-endothelial cell activity in the removal and storage of bromsulphalein in animals with total bile stasis. On the other hand, Rosenthal and Lillie²⁴ found that splenectomy and reticulo-endothelial blockade with colloidal quartz had no demonstrable effect upon bromsulphalein excretion in rabbits, suggesting that the reticulo-endothelial system plays no part in this process and implying that the polygonal cells of the liver are the elements chiefly concerned in the removal of the dye from the blood. This controversial point requires further investigation.

There is some evidence, both direct and indirect, that members of the phenolphthalein group of compounds may be destroyed in the tissues. Kendall²⁵ has shown that the disappearance of a portion of

injected phenolsulphonephthalein may be accounted for in this way, and Snell, Greene and Rowntree⁵ found that phenoltetrachlorophthalein is rapidly destroyed when incubated with ground liver or muscle tissue. Careful examination of animals with common duct ligation has frequently failed to reveal any evidence of the accumulation of the dye in the tissues in spite of the fact that only small quantities have been eliminated in the urine and little or none may be present in the blood. It seems likely that the existing wide variations in the degree of bromsulphalein retention in cats with total bile stasis may be largely dependent upon a variable rate of destruction of the dye in the tissues, although the possible importance of renal elimination and reticulo-endothelial storage cannot be denied.

SUMMARY AND CONCLUSIONS

1. Morphological changes in the liver and bile ducts are presented in relation to simultaneous changes in serum bilirubin concentration and bromsulphalein retention in 29 adult cats with uncomplicated total bile stasis produced by ligation of the common duct. The progression and regression of characteristic lesions in the liver and bile ducts are described in detail. The time of appearance of white bile is apparently extremely inconstant.

2. There was a marked degree of individual variation in serum bilirubin concentration. The highest incidence of maximum bilirubinemia in the group as a whole occurred early in the 2nd week of stasis, with a subsequent decline during the remainder of the experimental period (16 days). The time of occurrence of the initial fall in serum bilirubin concentration approximately coincided with the time of disappearance of mitotic figures from the hepatic cells, but this correlation was not consistent in individual cases.

3. There was no demonstrable correlation between the morphological changes and the serum bilirubin concentration at any given time during the period of total bile stasis.

4. The range of individual variation in the degree of bromsulphalein retention was more marked than in that of bilirubinemia and was not consistently related to the duration of stasis or to the serum bilirubin concentration.

5. There was no apparent correlation between the degree of dye retention and the morphological changes in the liver and bile ducts, either in individual instances or in the group as a whole.

'6. Anemia, activity of hemoglobin regeneration and suppression of bile acid synthesis are discussed in relation to their possible influence upon the changes in serum bilirubin concentration during total stasis. The observed variations in the degree of bromsulphalein retention may be dependent upon several variable factors, including destruction, storage or extrahepatic elimination of the dye.

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SIDEROTIC NODULES (GANDY-GAMNA BODIES) IN PRIMARY RENAL CARCINOMA *

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Since the first description of siderotic nodules appeared in 1904 (Stengel¹), these lesions are being observed with increasing frequency in the spleens of individuals presenting a wide variety of clinical conditions. They have also been encountered in the ovary (Henke and Lubarsch,² Kraus,³ Abrikossoff,⁴ and Kauder⁵), in hyperplastic thyroid tissue (Schuppiesser,⁶ and Kraus³), in the midbrain (Herzenberg⁷) and retroperitoneal lymph nodes (Schuppiesser,⁶ Kauder⁵) in cases of hemochromatosis, and in the bladder in a case of acute fulminating hemorrhagic and necrotizing cystitis (Kohly⁸). Their presence in other organs and tissues of the body, aside from the spleen, however, is rarely noted and only one report (Borromeo⁹) of their occurrence in the tissue of primary renal carcinoma could be found in the literature.

DESCRIPTION OF HISTOLOGICAL PREPARATIONS

The sections stained with hematoxylin and eosin consist of an area of kidney with an attached, solid, cellular, encapsulated carcinoma of the so-called hypernephroma type without much stroma or reduplication of cords, alveoli, tubules or cysts (Fig. 1). The renal tissue contains no pigment and shows only anemia with mild parenchymatous degeneration of tubular epithelium, except in the area adjacent to the capsule of the tumor where the atrophic and reactive changes usually observed under these circumstances are present. The cells of the tumor are large, polyhedral and uniform in size with finely and coarsely vacuolated cytoplasm, and regular, small, round, well stained nuclei which are rarely seen in mitosis. They are everywhere supported by a fine reticulum and further separated into sheets and masses by narrow bands and islands of vascular adult connective

* Received for publication January 4, 1935.

tissue. They are nourished in addition by blood carried in numerous, large, irregular, immature vessels consisting merely of clefts in the tissue lined by tumor cells. Macrophages with foamy or pigmented cytoplasm are occasionally observed engulfing erythrocytes and necrotic tumor cells.

The capsule separating the carcinoma from the renal tissue proper consists of a zone of connective tissue approximately 2 mm. wide and moderately cellular, but with very little pigment on the side toward the renal tissue. The remaining half of the capsule is composed of acellular, wavy collagen fibers arranged in bundles and diffusely impregnated in large patches with amorphous granules and variably sized, small, round, sharply etched bodies 8 to 15 microns in diameter which take hematoxylin. One corner of the capsule lying against the tumor tissue contains extravasated erythrocytes, carcinoma cells, lymphocytes, many blood vessels and peculiar pigmented lesions (Fig. 1).

The pigment occurs in the form of purple masses and coarse brown granules, and in concentric circular collections of slender, dark blue streaks and greenish, segmented, bamboo-like rods presenting a wreath-like arrangement about the blood vessels, not only in the capsule but also in the tumor tissue proper. In a typical example (Fig. 2) the center consists of a small vessel open to the flow of blood and lined by endothelial cells attached to a narrow wall of collagen material. This is surrounded by a wide, clear, foamy reticulated zone of delicate collagen fibers devoid of iron pigment and staining light blue with Mallory's and faintly red with Van Gieson's connective tissue stains. The outer limits of this zone end abruptly against a thin, wavy, and sometimes linear lamina, pale green in unstained preparations, dark blue with Mallory's potassium ferrocyanide stain and with hematoxylin and eosin, red with the fibroglia and Van Gieson's stains and black with Verhoeff's elastic tissue stain. Occasionally two lamina are present, separated by the interposition of thick, segmented and branched, bamboo-like rods, pale green in unstained preparations, brown and sometimes bluish with Mallory's collagen stain, bluish mustard-brown or brownish red with the fibroglia stain, blue with potassium ferrocyanide, green with hematoxylin and eosin, and greenish, greenish yellow and sometimes reddish with Van Gieson's stain. Although some of the vessels are surrounded exclusively by the bamboo-like rods, in the majority of instances the initial de-

posit of pigment occurs in the form of blue lines, the outer one of which is replaced by thicker, but similarly shaped bands of greenish material which ultimately develops into the bamboo-like rods. Eventually these segmented rods form confluent masses and their edges become progressively irregular and granular, nearing complete disintegration into amorphous *débris*. Many of the pigment masses are engulfed by foreign body giant cells and broken down in this manner. The giant cells form initially within the zone of foamy reticulated network and occasionally attain enormous proportions, having as many as seventy nuclei in single sections and many more in complete serial sections. As a rule they engulf only the pigment, leaving the thin-walled vessel and its surrounding reticulated zone intact; at other times, however, the whole vessel wall with its pigment and lumen together is engulfed. Within the giant cells the segmented rods and blue lamina are broken down into spicules, small crystals and rounded droplets.

The pigmentation within the tumor tissue proper presents a varied morphology. There are large bars of refractile, reddish brown, iron-free pigment unaffected by any of the staining reactions employed. The outlines of these bars are sharp and often appear freshly fractured; the surface is flat and seems to be constructed of layer upon layer of flat crystalline material resembling somewhat cholesterol plates. Other pigment masses are hyaline in character, stain pink with hematoxylin and eosin, red with Van Gieson's and blue with potassium ferrocyanide and Mallory's collagen stain. These hyaline masses occupy large areas traversed by thin strands of connective tissue and are closely related and sometimes continuous with denser, disk-shaped masses showing flat surfaces of concentric circles, often resembling small concretions (Fig. 3), and which stain an intense blue with potassium ferrocyanide, brown with Mallory's collagen stain and red, yellowish brown or even purple with Van Gieson's stain. The tumor cells in the vicinity of the immature blood spaces contain iron pigment which is found impregnating the chromatin material of the nucleus and which also occurs as small punctate dots or diffuse staining material in the cytoplasm. The reticulum supporting the tumor cells is stippled with fine, iron pigment granules contained within a poorly demarcated zone staining pink with eosin and somewhat orange with the fibroglia stain, and which appears like a neutrophilic smudge with hematoxylin and eosin. This fine, dust-like

pigment coalesces to form larger agminate masses, round or oval, with short projections or fine rosette formation, pale green in unstained preparations, green and sometimes pink with Van Gieson's stain, blue or mustard-brown with the fibroglia stain, and brown, brownish green or blue with Mallory's collagen stain. These masses sometimes seem to form in larger, hyaline, disk-shaped bodies. The smaller particles of pigment are phagocytosed by macrophages and in the larger islands of connective tissue which stud the tumor, there are enormous quantities of pigment (Fig. 4) often within giant cells or free in the form of a coarse feltwork traversed by connective tissue and staining various shades between blue and green in the potassium ferrocyanide preparations and green, brown or purple with hematoxylin and eosin. In these situations the giant cells contain irregular segmented rods with lateral projections and collateral off-shoots which serve to unite several of the masses into a single continuous piece.

DISCUSSION

A purely mechanical origin for the development of siderotic nodules was first postulated by Gandy,¹⁰ who attributed a great deal of importance to vascular stasis, hemorrhage, pigmentation and fibrosis. The relation between incrustations of calcium and iron salts on degenerated tissue fibers has recently been reviewed and discussed at length by Bennett,¹¹ and Davis and Warren.¹² Other investigators of these lesions have been concerned not only with hypothesizing an etiological basis for their development, but also in explaining the microscopic structure, the origin of the associated hemorrhages and the chemical nature of the pigments and other inorganic material of which they are composed. The characteristically shaped, pale green, pigmented rods which resemble mycelial structures are largely composed of iron phosphate (Kraus) deposited on a substratum of elastic tissue and collagen fibers (Glasunow,¹³ and Schuppiesser⁶). According to Christeller and Puskeppelies¹⁴ the intima and collagenous mural fibers of the smaller splenic arteries undergo degeneration with resulting extensive extravasation of blood which slowly hemolyzes, the liberated pigment becoming incrustated on the previously damaged fibrillar material. Gáspár,¹⁵ however, in agreement with most other observers, believes that in the spleen the lesions are derived from hemorrhages originating mainly in the vicinity of the trabecular

veins, the incrustations of the arterial walls within the area of involvement being secondary manifestations, which occur at first only on the side facing the hemolyzing erythrocytes. Fasiani and Oselladore¹⁶ effected the development of siderotic nodules experimentally in dogs and cats by ligating the veins at the hilum of the spleen, within which areas of necrosis were then produced by employing various physical and chemical agents such as caustics, diathermy coagulation and intraparenchymatous injections of alcohol and solutions of calcium chloride. Although emphasizing the importance of necrotic lesions in the genesis of these pigmented nodules, these investigators were inclined to support Jäger's¹⁷ view that the nodules can occur as a result of chronic stasis alone. If this were true, however, the conditions present in the neoplastic tissue of primary carcinoma of the kidney would constitute an ideal situation for their development, since the renal vein is frequently partially or completely occluded by proliferating tumor cells, and the occurrence of congestion, hemorrhage and necrosis is the rule. Only one case similar to our own has been reported in the literature (Borromeo⁹). Moreover, we were unable to find siderotic nodules in the histological sections of 62 additional cases of primary renal carcinoma in which, however, rapid cellular division, vascular thrombosis, passive congestion, hemorrhage, areas of necrosis, crystalline lipoidal material and the salts of calcium and iron were present in varying degree and combination. Although changes of this type may occupy an important secondary position in initiating the formation of siderotic nodules, it would appear that the genesis of these lesions depends primarily on the presence of additional factors, the nature of which is incompletely understood at the present time. Furthermore, these stimuli are evidently not operative in situations in which calcification commonly occurs, as for example, old infarcts, sclerotic blood vessels, fibrotic heart valves, areas of caseous necrosis and collections of inspissated pus.

In regard to a possible parasitic origin which has been postulated by Gibson,¹⁸ Nanta, Pinoy and Gruny,¹⁹ Askanazy and Schweizer,²⁰ and others, McNee²¹ believes that in view of the different organisms obtained in the reports just mentioned and in the cases examined by Jaffé and Hill,²² it would be illogical at present to regard the siderotic nodules as specific lesions resulting from mycotic infection. Cultures were not made in our case but there was no histological evidence in-

dicating the presence of parasitic infection. The statement made by Kohly that the mycelial-like threads in the siderotic nodules of splenic tissue represent multiple fractures of the rod-shaped elements by contractions of that organ does not seem tenable, in view of the fact that in the kidney of our case the segmented appearance was quite characteristic in the absence of any such contractile force.

SUMMARY

A description of siderotic nodules (Gandy-Gamna bodies) occurring in 1 of 63 cases of primary renal carcinoma is given and illustrated.

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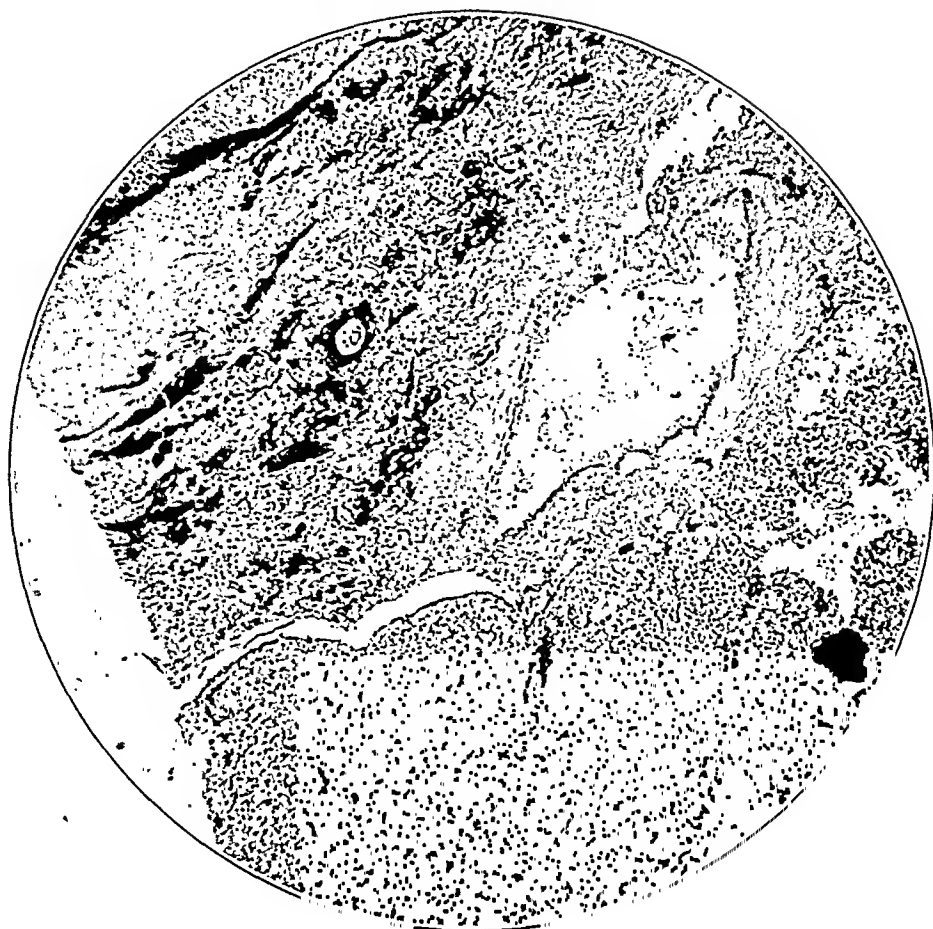
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DESCRIPTION OF PLATES

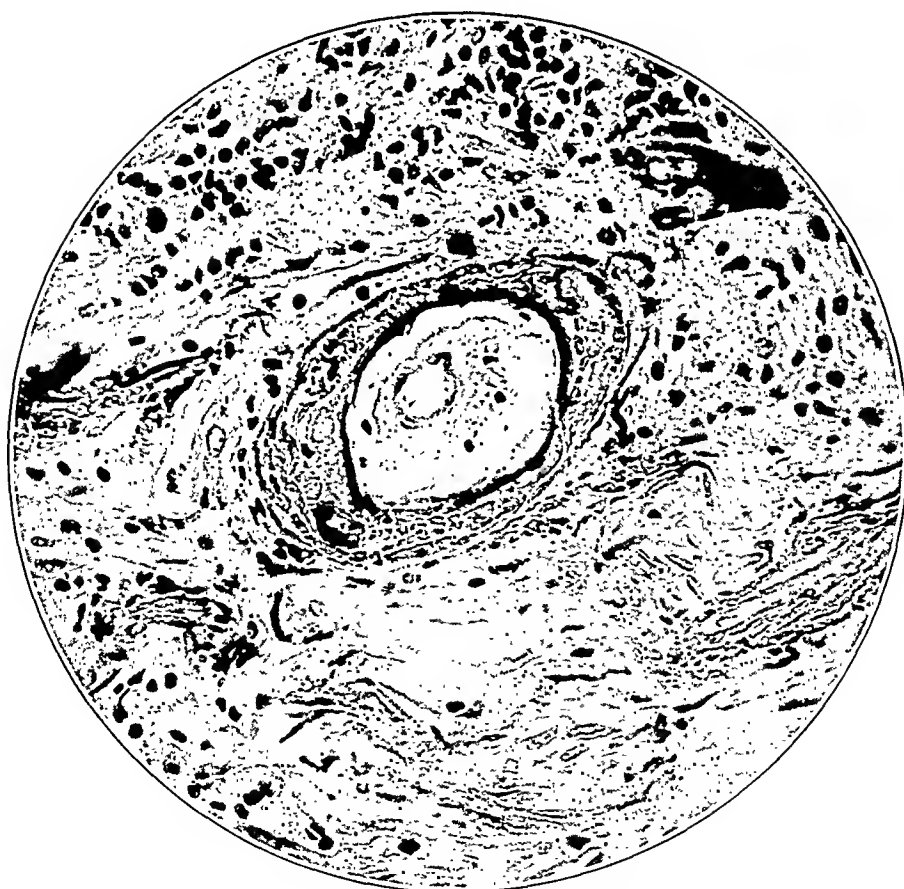
PLATE 73

FIG. 1. Encapsulated portion of primary renal carcinoma showing Gandy-Gamna bodies in upper portion of the field. Hematoxylin and eosin stain. $\times 50$.

FIG 2. Capsule of tumor containing a vessel surrounded by a dark wavy line and concentrically arranged, pale, segmented rods. The lumen of the vessel is narrow and is separated from the pigment by a fine reticulated structure containing a few nuclei. Note the presence of pigment in the surrounding tissue. Hematoxylin and eosin stain. About $\times 400$.



1

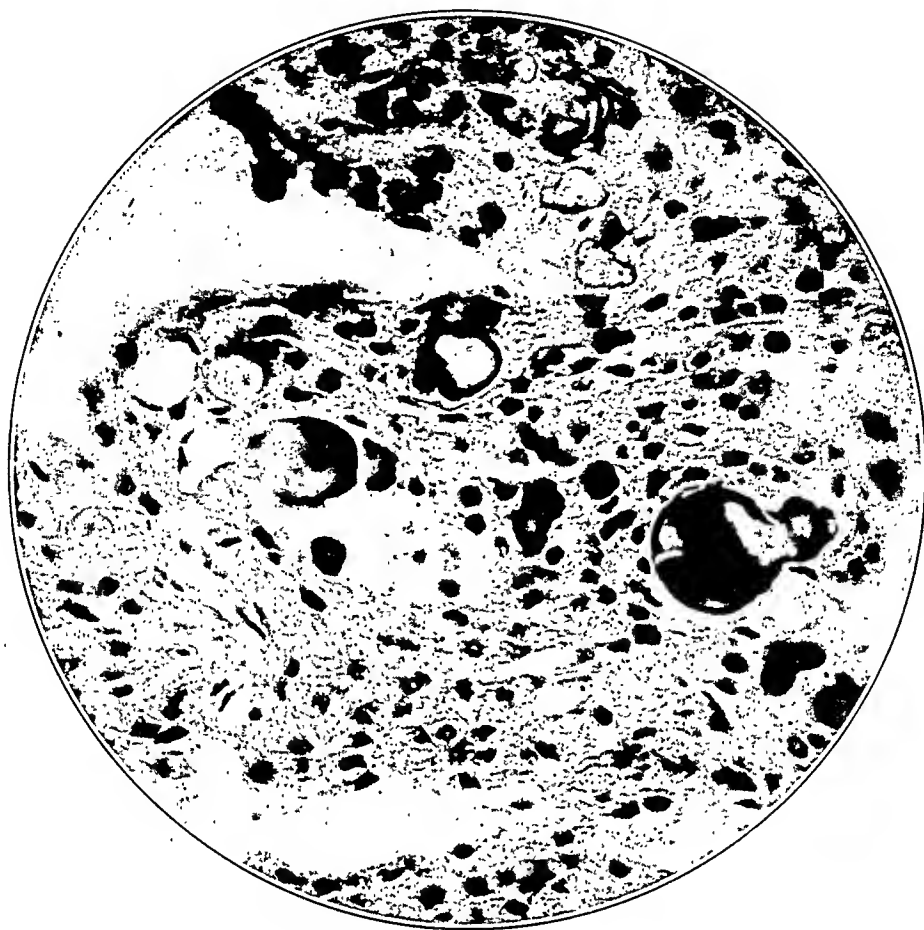


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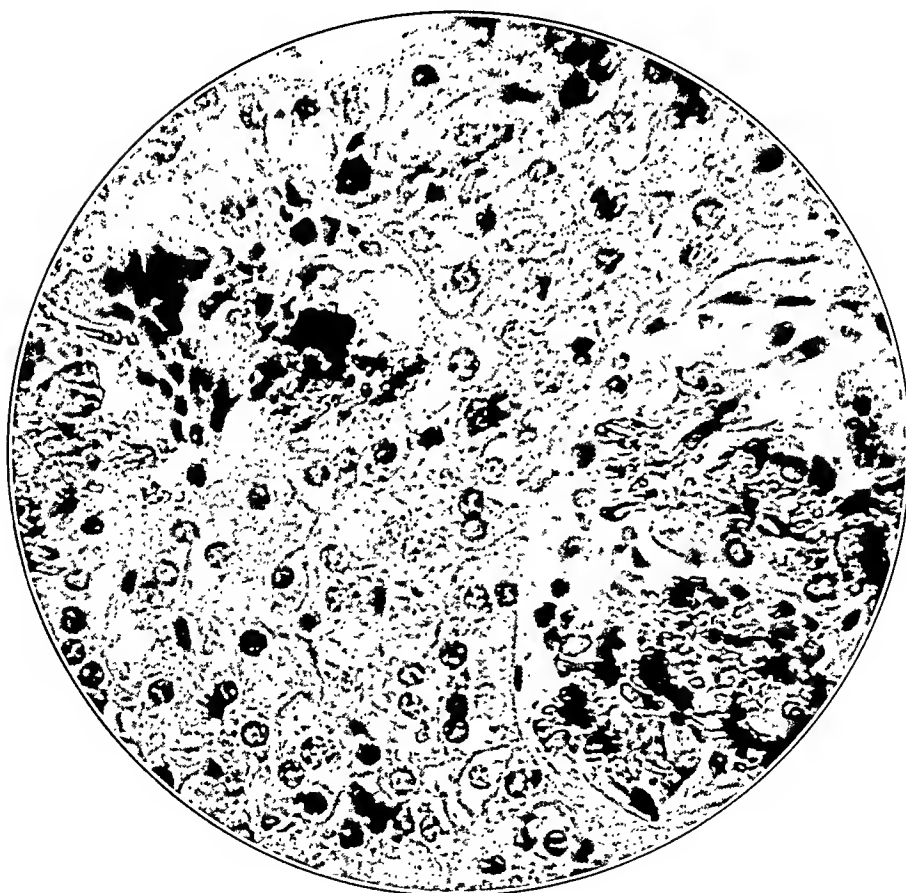
PLATE 74

FIG. 3. Disk-shaped pigment masses resembling small concretions. Hematoxylin and eosin stain. About $\times 450$.

FIG. 4. Island-like collections of pigment within the tumor tissue. Note the foreign body giant cell to the left of the center. Hematoxylin and eosin stain. About $\times 380$.



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THE AMERICAN JOURNAL OF PATHOLOGY

VOLUME XI

JULY, 1935

NUMBER 4

PRIMARY MELANOSARCOMA OF THE LEPTOMENINGES *

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Melanomas are malignant tumors arising in those regions where melanin-bearing cells are present. The usual sites are the pigmented nevi in the skin, the uveal tract of the eye, the pigmented layer of the retina, the adrenal gland, various pigmented regions of the mucous membranes and the meninges of the brain.

Primary melanoma of the meninges is considered to be rare and only 29 cases have been reported. A review of the literature reveals cases reported by the following authors: Virchow,¹ Sternberg,² Stoerk,³ Hirschberg,⁴ Pick,⁵ Minelli,⁶ Boit,⁷ Thorel,⁸ Bösch,⁹ Lindbom,¹⁰ Lua,¹¹ Hesse,¹² Esser,¹³ Schopper,¹⁴ Koelichen,¹⁵ Kiel,¹⁶ Matzdorff,¹⁷ Neubürger,¹⁸ Omodei-Zorini,¹⁹ Schmid,²⁰ Baumecker,²¹ Dieckmann,²² Farnell and Globus,²³ Foot and Zeek²⁴ (2 cases), Heilmann,²⁵ de Blasi,²⁶ Lackerbauer,²⁷ Garcin and associates,²⁸ and Jacob.²⁹ In a review of the reported cases Weimann³⁰ found that only 4 could be considered definitely to be primary melanosarcoma of the brain. Omodei-Zorini¹⁹ in his critical study of 20 cases could find only 12 certain cases.

The case reported here showed a diffuse melanosarcomatosis arising primarily in the pia with secondary involvement of the brain parenchyma. The histological study of the process in the leptomeninges and the brain revealed that the various cells of the neoplasm belonged to various developmental stages in the formation of the chromatophore.

* Aided by a grant from the Fluid Research Fund contributed by the Rockefeller Foundation.

Received for publication February 13, 1935.

REPORT OF CASE

Clinical History: On May 30, 1932, a white male, aged 82 years, was admitted to the Strong Memorial Hospital in a comatose condition. According to his family, the patient had enjoyed excellent health except that for the past few months he had been subject to dizzy spells. Thirty-six hours before admission he complained of feeling ill, a few minutes later he lost consciousness and had a clonic convulsion involving the left arm and leg.

Physical Examination: The patient was a well developed, poorly nourished, elderly man in a comatose state. The hands and lips were cyanotic, and the skin was cold and clammy. The respirations were stertorous, shallow and rapid. The temperature was 40.7° C. by rectum, the pulse 58 and respirations 52.

Neurological examination disclosed a conjugate deviation of the eyes to the left and the pupils contracted and fixed to light. The fundi showed arteriosclerosis, but no blurring of the optic discs. The extremities were flaccid and the deep tendon reflexes were absent bilaterally. Plantar stimulation resulted in dorsal flexion of the large toe on the left side and there was a positive Hoffmann's sign on the left side.

Course of Illness: The patient did not regain consciousness and died 1 hour after admission.

Clinical Diagnosis: Intracranial hemorrhage in the right internal capsule.

AUTOPSY REPORT

The autopsy was performed 14 hours after death. Except for the brain, no abnormal pigmentations over the body or in the internal organs were found. Examination of internal organs showed generalized arteriosclerosis and bronchopneumonia.

The brain weighed 1500 gm. and was of normal consistence with moderate widening of the sulci over the frontal lobes. There was a moderate thickening of the larger cerebral arteries and the blood vessels in the Rolandic fissure were greatly engorged on the right side.

The remarkable feature was the extreme brownish black pigmentation which was symmetrically but unevenly distributed in the leptomeninges on both sides of the brain. The pigment was most intense around the Sylvian and Rolandic fissures, extending far out over the frontal and parietal lobes but diminishing as the frontal and occipital poles were reached. On the mesial surface and base of the brain the pigmentation was much less marked. As the pigmentation diminished in amount it was arranged in discrete flecks of various sizes measuring from 0.5 to 5 mm. in diameter (Fig. 1). Where the meninges were stripped from the underlying brain tissue a few small gray to black dots measuring up to 2 mm. were seen to invade the

cortex. After fixation in formalin careful sectioning disclosed in all only four small, dark, circumscribed nodules extending from the meninges into the cortex of the cerebrum. These nodules were found in the pars opercularis of the gyrus frontalis inferior, the pars superior of the gyrus frontalis medius (two nodules) and the area parolfactorius of the right side. Macroscopically this invasion was limited to the gray matter and did not extend into the medullary layer.

The ventricular surfaces were free of pigment with the exception of the right wall in the recessus suprapinealis and along the tela choroidea ventricularis where the melanosis extended up to the foramen of Monro, and a few small discrete flecks in the right lateral ventricle.

The spinal cord was not removed except for a small portion of the upper cervical cord.

MICROSCOPIC EXAMINATION

Pieces of tissue were taken from various parts of the central nervous system, including especially those regions in which the brain parenchyma was invaded by the neoplastic process. The stains employed were thionin, hematoxylin-eosin, Van Gieson, Globus' modification of Cajal's method for astrocytes, Penfield's modification of Hortege's method for microglia and oligodendroglia, Weil's myelin stain, Herxheimer's scharlach R stain for fat, Marchi's stain, and Perdrau's stain for connective tissue. The pia over the gyri and in the sulci was carefully stripped off and various silver methods were employed to stain the nerve endings. The most satisfactory method was found to be Snessarew's modification of Cajal's technique.³¹

The pigment showed the reactions characteristic of melanin. The granules were completely decolorized by hydrogen peroxide, nascent chlorine and 2 per cent chromic acid. Similarly, treatment of sections with 0.5 per cent potassium permanganate for 24 hours, followed by 0.5 per cent oxalic acid, resulted in complete bleaching of the melanin granules. Concentrated solutions of acids and alkalis and the ordinary fat solvents did not dissolve the granules. The pigment gave no reaction with scharlach R and tests for iron were negative. In unstained sections the granules were light to dark brown in color; with thionin the granules were dark green; in hema-

toxylin-eosin and Van Gieson stains the granules were light to dark brown, and with silver stains the granules were black. The silver stains also brought out the granules in greater abundance in all types of pigment cells.

Meninges: The dura was free of melanin-containing cells. The leptomeninges were as a rule not thickened and no inflammatory cells were seen. In every section that contained meninges chromatophores were found (Fig. 2). Most frequently the chromatophores were in single layers and limited to the pia intima and the pial lymph space about the blood vessels. However, in those regions where the pigment flecks were present the chromatophores were sometimes several layers in thickness. In such areas it was not infrequent to find that these pigmented cells had invaded the brain parenchyma by way of the perivascular spaces (Fig. 3). The arachnoid contained chromatophores only in those regions where the pigment flecks were large and active proliferation of cells was apparently taking place.

In the strips of pia the pigmentation was found to be variable. Those areas that were relatively unpigmented showed typical chromatophores, isolated or arranged in groups. In the latter the processes of individual cells appeared to anastomose freely, producing a so-called chromatophore net. In general, the melanin granules were usually found within cells, but occasionally pigment was seen lying free in the pia. The chromatophores usually had a very definite relation to the blood vessels, as may be seen in Figure 4. In this illustration the round pigment cells are arranged around the walls of the blood vessels. These cells are limited to the membrane forming the pial lymph space but do not invade the adventitia. In other sections the chromatophores were arranged in such a manner that their processes ran parallel with the course of the blood vessels.

The pigment flecks showed, under low magnification, a dark center with radiating processes going off from the core very much like the spokes of a wheel (Fig. 5). Under higher magnification the center was found to consist of numerous small, round pigmented cells. The radiations were made up of several different types of pigment cells (Fig. 6). Consequently, five types of pigment cells could be differentiated as follows:

(1) Round cells which measured 8 to 12 μ in diameter. These cells were most numerous and were found usually in the center of

the pigment fleck. The melanin was in the form of minute granules which were of a light brown color in unstained sections. The nucleus was vacuolated and centrally placed; occasionally double nuclei were seen.

(2) Oval cells with and without short plump processes. The cell body had an average size of 15 by 10 μ . These cells were fairly numerous and were found most frequently in the immediate periphery of the core and occasionally within the core. The granules were identical with those found in the round cells. The nuclei were vacuolated and centrally placed. These cells were apparently transitional forms between the round pigment cells and the spindle-shaped chromatophores with long processes (Fig. 7).

(3) Spindle-shaped chromatophores. The average size of the cell body was 30 by 10 μ . These cells formed the most numerous proportion of the chromatophores and usually possessed two long delicate processes which sometimes attained a length of 200 μ . They were found in the periphery of the pigment fleck and the relatively unpigmented areas. The granules were fairly numerous and of a darker brown color than those seen in the round or oval cells. The nucleus was round and vacuolated. Occasionally, giant types were seen whose cell body measured 70 by 30 μ and whose processes were 300 μ in length. In these cells double and triple nuclei were frequently found.

(4) Polyhedral cells with and without short plump processes. The average size of the cell body was 15 by 12 μ . These cells were few in number and were found in those areas where the oval cells with short processes occurred. The melanin granules were identical with those found in the round cells. The nucleus was vacuolated and centrally placed. Much larger cells containing two or more nuclei were rarely seen. The general impression obtained was that these cells were transitional forms between the round cells and the typical stellate-shaped chromatophores (Fig. 8).

(5) Stellate-shaped chromatophores. The average diameter of the cell body was 20 μ . These cells were relatively few in number. As in the case of the mature spindle-shaped chromatophores, these cells were found in the periphery of the pigmented flecks and in the relatively unpigmented areas. They contained various numbers of processes, the usual number being four. The processes were long and delicate, and sometimes reached a length of 150 μ . The granules

were similar to those found in the spindle-shaped chromatophores. The nuclei were round and vacuolated and usually centrally placed. Giant stellate cells were rarely seen. The body of these cells was very large with a diameter of $50\ \mu$, and the processes occasionally reached a length of $200\ \mu$. Double nuclei were infrequently seen.

The pigment flecks in the walls of the third and right lateral ventricle were similar histologically to those found in the pia.

Nerve Endings of Pia: Numerous strips of pia taken from various regions were stained for nerve endings in order to study the possible relation between them and the pigment flecks. None was discovered. The most frequent type of nerve ending seen is illustrated in Figure 9.

Parenchyma: The nerve cells appeared to be fairly well preserved in thionin-stained sections, but in scharlach R fat stains numerous small fat globules were seen in the cytoplasm. This "lipoid degeneration" was found throughout the entire cortex but was most marked about those regions where the neoplastic process had extended into the brain parenchyma. In the periphery of the invading tumors the nerve cells contained melanin granules in various amounts. In the frontal and the temporal lobes there was a moderate degree of sclerotic change in the nerve cells of the third layer. In the hippocampus numerous small, acellular areas were found, the so-called "Verödungsherde." The nerve cells in the lenticular nucleus and thalamus contained moderate amounts of lipochrome. The Purkinje cells of the cerebellum were well preserved and free of lipochrome pigment. The cells of the substantia nigra and locus cæruleus appeared normal and contained normal amounts of large melanin granules.

The astrocytes were not hypertrophied or hyperplastic. In those regions where the neoplastic process had invaded the parenchyma the protoplasmic and fibrillary astrocytes contained melanin granules within their cytoplasm and in their processes. The oligodendroglia were free of pigment granules. The microglia showed various stages of pathological change. Some appeared normal, others contained occasional melanin granules in their processes, while others were so completely filled with melanin pigment that they were globular in form and looked very much like the round pigmented cells found in the pigment flecks of the pia and in the tumor nodules of the parenchyma. The pigment cells could be differentiated from

the "Gitterzellen" by the fact that the former contained a vacuolated nucleus and the cytoplasm was free of fat. In the brain substance the blood vessels were increased in number and engorged about those regions where the tumor had invaded the parenchyma. The arteries showed moderate arteriosclerosis and calcification of the blood vessel walls occurred in the hippocampus and the lenticular nuclei. Occasionally, minute melanin granules were found in the endothelial cells of the capillaries. No melanin-containing cells were found within the lumen of the blood vessels.

Stains with Marchi and scharlach R showed degeneration in the medullary layers where the cortex was involved by the tumor. No degeneration was found in the pyramidal tracts with myelin or fat stains.

The cranial nerves were intact. The pia encircling the nerves was frequently outlined by chromatophores, but no infiltration into the epineurium was observed.

Tumor in the Parenchyma: The involvement of the parenchyma by the neoplastic process was minimal in contrast to the diffuseness of the process in the meninges. Microscopic study revealed many more areas of invasion of the cortex than could be seen macroscopically. These nodules were deeply pigmented and in spite of much search no unpigmented nodules were found.

In view of the fact that all the nodules were identical in structure, a description of the largest one is deemed sufficient (Fig. 10). The infiltrative nature of the neoplasm by way of the perivascular spaces is well illustrated and the process is limited to the cortex. However, the perivascular manner of distribution cannot explain the whole means by which this invasion occurred since pigment cells were found lying free in the brain parenchyma in the peripheral regions of the tumor (Fig. 11). Apparently the pia-glia membrane was not an impenetrable barrier against the tumor cells, for frequently a heavily pigmented cell with a round vacuolated nucleus was found in the act of passing through the membrane, half of the cell being in the perivascular space and the other half in the parenchyma. Occasionally, isolated spindle-shaped chromatophores were found lying free in the parenchyma in no close proximity to blood vessels whose perivascular spaces were filled with melanin cells.

The general arrangement of the pigmented cells in the tumor suggested a typical melanosa sarcoma. The spindle-shaped cells were

arranged in bundles going in all directions, so that transverse, longitudinal and oblique aspects of these sheaves of cells could be seen. Not infrequently whorls of compact masses of spindle cells were observed (Fig. 11). Among these bundles of pigmented spindle cells were found round, oval and polygonal pigmented cells. In the more peripheral regions of the tumor the pigment cells formed a ring several layers thick about the blood vessel and definite invasion of the adventitia by the chromatophores occurred (Fig. 11). In teased preparations from the nodule all the types of pigment cells found in the pia were seen (Fig. 12).

With connective tissue stains, such as Van Gieson's and Perdrau's, typical reticulum and collagen fibers were found in large numbers throughout the tumor (Fig. 13).

DISCUSSION

Occurrence of Chromatophores in the Meninges

Chromatophores in the leptomeninges were, according to Virchow,¹ first described by Valentin. Since then Kölliker,³² Obersteiner,³³ Charpy,³⁴ and others have described the occurrence of these chromatophores in the meninges of normal adult brains. Obersteiner³³ described these cells in the adventitia of the blood vessels and pia of the medulla oblongata and could trace these chromatophores in the pia around the blood vessels into the brain substance. Kölliker³² and Golman³⁵ insisted that the pigmentation was limited to the pia. The usual sites of these chromatophores are in order of frequency: base of the medulla oblongata and cerebellum, about the dorsal surface of the upper cervical region, base of the cerebral peduncles, region of the optic chiasma, and along the sylvian fissure and the inferior surfaces of the frontal, temporal and occipital lobes.

Regarding the appearance of chromatophores of the pia in relation to age, Broniatowski³⁶ studied the pia mater about the medulla oblongata in a series of patients from the ages of 3 to 71 years. He found that melanin-containing cells appear at the age of 9 years and that after puberty they do not increase in number but that the color of the granules changes from a light brown to a dark brown. Farnell and Globus²³ state that they have frequently seen chromatophores in infants and have found them in a fetus of 5½ months. The relation of pigmentation of the pia to race has been mentioned

infrequently. Mohnike³⁷ noted the presence of pigment in the leptomeninges of the brain in the Javanese, but did not find it in the negro. According to Freeman,³⁸ chromatophores are more numerous in the brains of negroes than in those of the white race. Virchow stated that pigmentation of the base of the brain was present in all normal adults of the Caucasian race. Symmers³⁹ reported excessive pigmentation of the pia in more than half of a series of 177 routine autopsies on Egyptians.

The chromatophores of the pia can become hyperplastic, leading to a diffuse melanosis of the pia, and the pigment flecks are often symmetrically arranged on both sides of the brain. In the gray horse (Virchow), calf (Casper), and sheep (Schwalbe and Weidenreich), excessive pigmentation of the meninges is not uncommon and is usually associated with abnormal pigmentation of the skin. According to Dawson,⁴⁰ melanomas of the meninges have been described in the horse and sheep. In man, Rokitansky,⁴¹ Oberndorfer,⁴² Grahl,⁴³ Maclachlan,⁴⁴ and Berblinger⁴⁵ have described cases of marked pigmentation of the brain allied with numerous pigmented nevi of the skin. Similarly, in the cases of primary melanoma of the meninges reported by Esser,¹³ Lua,¹¹ Lindbom,¹⁰ and Schopper,¹⁴ numerous nevi in the skin were present. The presence of developmental disturbances in cases of abnormal melanotic pigmentation and melanoma of the meninges was reported by Hamill and Rothstein⁴⁶ (syringomyelia), Koelichen¹⁵ (syringomyelia), Bösch⁹ (multiple sclerosis), and Berblinger⁴⁵ (glioma).

According to Spielmeyer,⁴⁷ under various chronic pathological conditions and in senility the number of chromatophores increases. General paresis is the condition most apt to produce this increase and in chronic encephalitis involving the cells of the substantia nigra the amount of melanin may be greatly increased in the pia about the cerebral peduncles. Jakob⁴⁸ states that in any chronic irritative condition of the pia a marked hypertrophy and hyperplasia of chromatophores may occur. This excessive pigment is found in the pia, the fibrous neuroglia and the microglia. In the glia cells the pigment is in the processes in the form of large granules, but in the microglia this pigment is finely granular. The ganglion cells and oligodendroglia cells are free of this pigment. As a possible explanation of this hyperpigmentation the observation of Peck⁴⁹ may be utilized. He has shown experimentally that the increased pigmen-

tation in the skin associated with inflammation is related to a sudden and strong dopa reaction, even in the absence of light.

It is a well known fact that hyperpigmentation of the skin occurs in Addison's disease. Similarly, Fagge⁵⁰ finds that the pigmentation of the pia may be greatly increased in this disease also. This may be explained in the same manner that Bloch⁵¹ explains this condition in the skin. Due to the failure of the adrenal glands to synthesize adrenalin from its mother substance, an excess of this normal precursor circulates in the blood. This precursor is closely related to 3-4 dihydroxyphenylalanine (dopa), possibly identical with it. Circulating in the blood, it is possible that the excess of this adrenalin precursor on reaching cells in the pia is acted on by the contained melanin-forming ferment (dopa oxydase) and oxidized into melanin.

Origin and Nature of the Chromatophore

Active debate has arisen regarding the origin and nature of the chromatophore. In the lower animal kingdom most authorities look upon the chromatophore as a specialized cell which is derived originally from the mesoblast and which retains an intimate relation with the nervous system (Ballowitz,⁵² and Königs⁵³). Stockard⁵⁴ believes that in batrachians these cells arise from the mesoblastic tissue in the ovum and embryo and remain distinct throughout the life of the animal. In man and the higher mammals Bloch⁵¹ distinguishes between the chromatophores and the melanoblasts. According to him, the melanoblast is capable of forming melanin pigment and is derived from ectodermal and mesodermal tissue. The ectodermal melanoblast is found in the basal layer of the epidermis, the follicles and matrix of pigmented hairs, in pigmented nevi, in the retina, and is probably identical with the dendritic cells found in the skin and mucous membranes of ectodermal origin. The mesodermal melanoblasts are found in the uveal tract of the eye, in the meninges and in the Mongolian spots or blue nevi. The chromatophore is, according to Bloch, a mesodermal cell merely containing melanin pigment which it has phagocytozed, but is itself incapable of forming pigment.

Bloch's theory of the chromatophore in the skin can be reconciled with the epithelial school of adherents who believe that the melanin is produced in the basal cells of the epidermis and is discharged and taken up or phagocytozed by mesodermal cells in the

dermis to form the chromatophore. In the meninges, however, this conception is difficult to accept unless we introduce the presence of epidermal embryonic rests or consider the melanin to arise from the cells of the substantia nigra, locus cæruleus, and so on. It is a well known fact, as mentioned previously, that the number of chromatophores around the cerebral peduncles may be increased in chronic encephalitis where the cells of the substantia nigra are affected. Dieckmann²² observed nevi in the pia mater and believed that they gave rise to the pigmented flecks in the meninges. According to him these nevi contain, besides spindle-shaped chromatophores, round and polygonal cells which may contain various quantities of melanotic pigment or may be completely free of pigment. Consequently, it is possible that these nevi may produce chromatophores, as Ewing⁵⁵ has demonstrated in the deep pigmented nevi of the skin. Ewing, however, cautions against too general an application of this origin of the chromatophore and believes that the adult chromatophores in the meninges have no connection with nevus cells.

Recently an increasing collection of evidence suggests the possibility that melanin production is always of neurectodermal origin. Weidenreich⁵⁶ expressed this idea when he said that "the primordial pigment cells arise from a detached portion of the neural tube." In the past the leptomeninges have been considered to be entirely of mesodermal origin as a result of the work of His,⁵⁷ von Kölliker,⁵⁸ and Weed.⁵⁹ The work of Oberling,⁶⁰ and Harvey and Burr,⁶¹ however, suggests that neurectodermal elements contribute to the formation of the meninges. This would suggest that neurectodermal tissue might form the pigment which would then be phagocytosed by mesodermal cells to form chromatophores.

Ribbert⁶² believes that the rigid distinction between chromatophore and melanoblast does not exist because under certain conditions the chromatophore can become active in the formation of melanin. He considers the chromatophore as a specially characterized cell of mesodermal origin. In the choroid of the eye, Miescher⁶³ observed that the dopa reaction is positive during a short period of embryonic life, coincident with the formation of pigment by the mesodermal melanoblasts, and that after birth these cells become dopa-negative. In other words, the chromatophores are mesodermal melanoblasts in a resting state. Under pathological conditions they

may resume their embryonic activity. Similarly, Bloch and his co-workers have shown that the melanin-containing cells in the meninges are dopa-positive in embryonic life but lose this reaction later. In all probability, therefore, the melanin in the chromatophores of the meninges is an autochthonous product and in the formative stages these chromatophores are true melanoblasts. Such an interpretation would agree with Stockard's observations.

Origin of Melanomas

The origin of melanomas is still in a highly controversial state and presents many perplexing problems. Dawson⁴⁹ has very ably reviewed this subject in his monograph. The school of Unna claims their origin from the epidermis. In recent years the theory of the nervous origin of nevi and melanomas has gained adherents. The mesodermal school is divided. Simon considers melanomas to arise from young undifferentiated cell forms; von Recklinghausen believes they arise from a proliferation of the endothelium of lymphatic vessels; Pick and Jadassohn believe they spring from the endothelium and perithelium of blood vessels; and Ribbert⁶² believes that melanomas arise from the chromatophores and consequently calls these neoplasms chromatophoromas.

The school believing in the epidermal or epithelial origin claims that the melanomas in the meninges arise from displaced embryonic ectodermal rests. Wieting and Hamdi⁶⁴ differentiated between two types of pigmented tumors: the chromatophoroma consisting of cells which phagocytose melanin secondarily, and melanoblastoma composed of cells which produce melanin. This school consequently considers all melanoblastomas to be of epithelial origin and many pupils of the ectodermal school would call these tumors melanocarcinomas, insisting that the chromatophoromas arise from cells which are capable only of taking in melanin pigment. Bloch⁵¹ does not see the need of such a distinction. According to him a melanocarcinoma arises from ectodermal melanoblasts and a melanosarcoma arises from mesodermal melanoblasts.

The nervous theory has been championed by Ewing.⁵⁵ Such a theory would explain very well the melanomas arising primarily in the adrenal gland since the chromaffin cells of the suprarenal medulla are of sympathetic origin and arise from the neurectoderm which gives rise to the ganglion cells and the sheath of Schwann cells.

Similarly, the findings of Oberling⁶⁰ and Harvey and Burr⁶¹ would suggest that a neurectodermal origin of melanoma in the meninges is possible. In 1882 von Recklinghausen⁶⁵ discussed the origin of nevi of the skin in relation to multiple fibromas of the skin and 17 years later Soldan⁶⁶ confirmed his findings and concluded that nevi were a phase of neurofibromatosis. He believed the nevus was nervous in origin since he found nerve fibrils among the nevus cells. Laidlaw and Murray⁶⁷ have proposed an ingenious hypothesis that the pigmented mole is related to the tactile spots of reptiles and amphibia. In his masterly work Masson⁶⁸ rediscovered Soldan's findings and elaborated upon them in detail. He found that the nevus cells were derived from specialized cells in the sensory nerve endings of the skin. The superficial pigmented nevus which occurs in the epidermis of the skin is derived from the epithelial-like cells of the Merkel-Ranvier body and the chromatophores or cells of Langerhans. These latter cells lie among the epithelial cells accompanying the neurofibrils and are capable of producing pigment. (Bloch, however, claims that these Langerhans cells are not melanoblasts but nerve cells.) Should this type of nevus undergo malignant changes it would be a melanocarcinoma. The deep pigmented nevus occurring in the dermis arises from the specialized cells in the core of the Meissner corpuscle. As in the case of the specialized cells of the Merkel-Ranvier body, the origin of these cells is in doubt, although most authorities who have worked on this subject believe these cells are mesodermal in origin. Consequently, a malignant neoplasm arising from a Meissner corpuscle would give rise to a melanosarcoma.

Stöhr⁶⁹ described occasional nerve endings in the pia which are very similar morphologically to the Meissner corpuscles found in the dermis. The close relation between the chromatophore and the nerve endings in the pia mater of man has been noted by Golman,³⁵ and Snessarew.⁷⁰ Dieckmann,²² and others, found nevi in the pia mater which bore a close resemblance to those seen in the skin. In each of the 2 cases of melanoma of the meninges reported by Foot and Zeek²⁴ they were able to find fibrils "in some way connected with peripheral nerves" in the tumors. Their findings led them to believe that melanomas are derived from the nerve endings in the pia.

Consequently, in the present study, strips of pia were stained for nerve endings in order to study the relation between the pigment

flecks and the nerve endings. No specific relation could be discovered, yet this does not preclude the possibility that the originally malignant pigment flecks might have arisen from a Meissner-like corpuscle in the pia.

The exact place of origin of the malignant neoplasm could not be found, as is usually noted in the cases reported in the literature. This may be understood on the basis that the meningeal involvement is apt to be diffuse as a result of the ease with which tumor cells can be disseminated over the brain by the constant flow of the cerebrospinal fluid in the subarachnoid space. It is not difficult to understand, therefore, that a diffuse melanosarcomatous infiltration of the meninges can occur without involvement of the cerebral parenchyma, as seen in the cases of Virchow,¹ Stoerk,³ Thorel,⁸ and Esser.¹³ The general impression of pathologists regarding diffuse tumors in the meninges and brain parenchyma is first that the primary seat of the tumor is in the meninges and secondarily that it invades the nervous parenchyma, as seen in the cases of Schopper,¹⁴ Sternberg,² Bösch,⁹ Matzdorff,¹⁷ and Lackerbauer.²⁷ I believe that the case reported here is similar since the involvement of the meninges was much greater than that of the parenchyma.

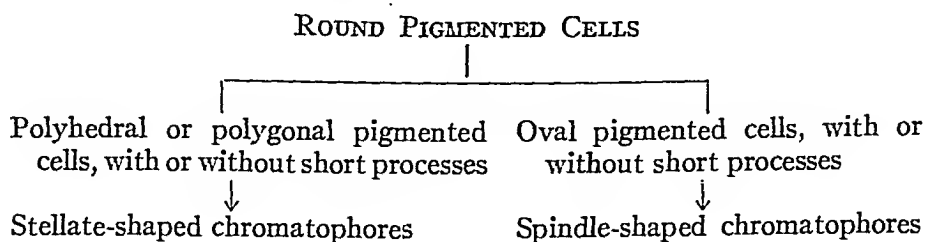
In view of the rather general belief that the meninges are of mesodermal origin, the followers of the mesodermal school have felt that the occurrence of primary melanoma in the meninges was a proof of their theory. Baumecker²¹ interpreted his findings as evidence that melanomas arise from the endothelial cells of the blood vessels. Many workers have noted the relation of pigmented cells to the blood vessels in primary melanoma of the brain, and Minelli⁶ states that such a tumor should be called a melanotic perithelioma. It is now generally accepted that there are no true lymphatic vessels in the brain and consequently the theory of von Recklinghausen could not be applied to primary melanoma of the brain.

The views of Ribbert⁶² have been most popular in interpreting the origin of melanoma in the brain. Virchow¹ found all types of transition from simple hyperplasia and hypertrophy of pigment cells to sarcomatous changes, and believed that the widespread melanosis was derived from the hyperplasia and hypertrophy of the chromatophores. The presence of unpigmented cells so often seen in melanomas is interpreted by Ribbert and Matzdorff¹⁷ as immature chromatophores. Hirschberg,⁴ Pick,⁵ Koelichen,¹⁵ Schmid,²⁹ Esser,¹³ and others believe melanomas arise from the chromatophores nor-

mally present in the meninges. In the cases of Boit⁷ and Lindbom,¹⁰ the tumors arose primarily in the dura mater and in view of the fact that chromatophores are never normally found in the dura, Boit believed that the chromatophoroma in his case originated from displaced chromatophores.

SUMMARY

A review of the literature of the various hypotheses regarding the origin and nature of the chromatophore of the leptomeninges leads me to conclude that chromatophores can become active in the production of melanin and give rise to melanoma. This is in agreement with Ribbert's view that no rigid distinction between melanoblast and chromatophore is possible. The chromatophores of the leptomeninges are very much like those found in the choroid of the eye; they are mesodermal melanoblasts in a resting state. In other words, the melanin in the chromatophores of the meninges is an autochthonous product and not the phagocytosed pigment produced by other cells. Under certain pathological conditions these chromatophores or dormant melanoblasts can resume their embryonic activity in the production of melanin and behave like true melanoblasts. From the present study similar conclusions can be drawn. The chromatophores normally present in the pia can take on the rôle of active melanin production. The formation of the chromatophore from an undifferentiated round pigment cell is suggested from a study of the structure of the pigmented flecks found in the leptomeninges. In the center of the fleck numerous, small, round pigmented cells were found. The periphery of this core consisted of polymorphous pigmented cells. In the most peripheral portions typical stellate and spindle-shaped chromatophores were found. Thus, the polymorphism of the pigmented cells in the tumor can be considered to be due to the fact that the various cells are merely different transitional forms in the development of the chromatophore. This development can be illustrated in the following way:



Matzdorff¹⁷ concluded that the round pigmented cell was an incomplete development of the chromatophore.

Similarly, it is apparent that the chromatophore in the pia, under certain pathological conditions, can take on malignant characteristics and give rise to a melanosarcoma. This is, I believe, what happened in the case here reported. One may call this tumor a chromatophoroma or a melanoblastoma. The general appearance of the tumor nodules is strongly suggestive of melanosarcoma.

No evidence could be found suggesting that the neoplasm may have arisen from nerve endings in the pia. The close relation between the chromatophores and the nerve endings in the pia mater of man normally is interesting. It is possible that the malignant condition arose from a Meissner-like corpuscle in the pia, but no evidence of such a phenomenon could be found in this case.

CONCLUSIONS

1. Chromatophores normally present in the pia can take on melanoblastic functions. It is suggested that the chromatophore is a resting mesodermal melanoblast.

2. Under pathological conditions, as in malignancy, the chromatophore can resume its melanoblastic activity and give rise to a primary melanosarcoma of the leptomeninges. This is, I believe, what occurred in the case reported here.

3. The development of typical stellate-shaped and spindle-shaped chromatophores from round pigment cells is suggested by study of the pigmented flecks in the pia.

4. The parenchymatous involvement was secondary and occurred by way of the perivascular spaces.

5. The various types of pigmented cells seen in the tumor nodules of the parenchyma were identical with those found in the pigmented flecks of the pia.

NOTE. I am grateful to Drs. George H. Whipple, William S. McCann, William B. Hawkins and Wilbur K. Smith for valuable advice. I also wish to express my great appreciation to Dr. George W. Corner, who kindly allowed me laboratory facilities.

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DESCRIPTION OF PLATES

PLATE 75

FIG. 1A. Lateral view of brain.

FIG. 1B. Mesial view of brain.



IA



IB

Akelaitis

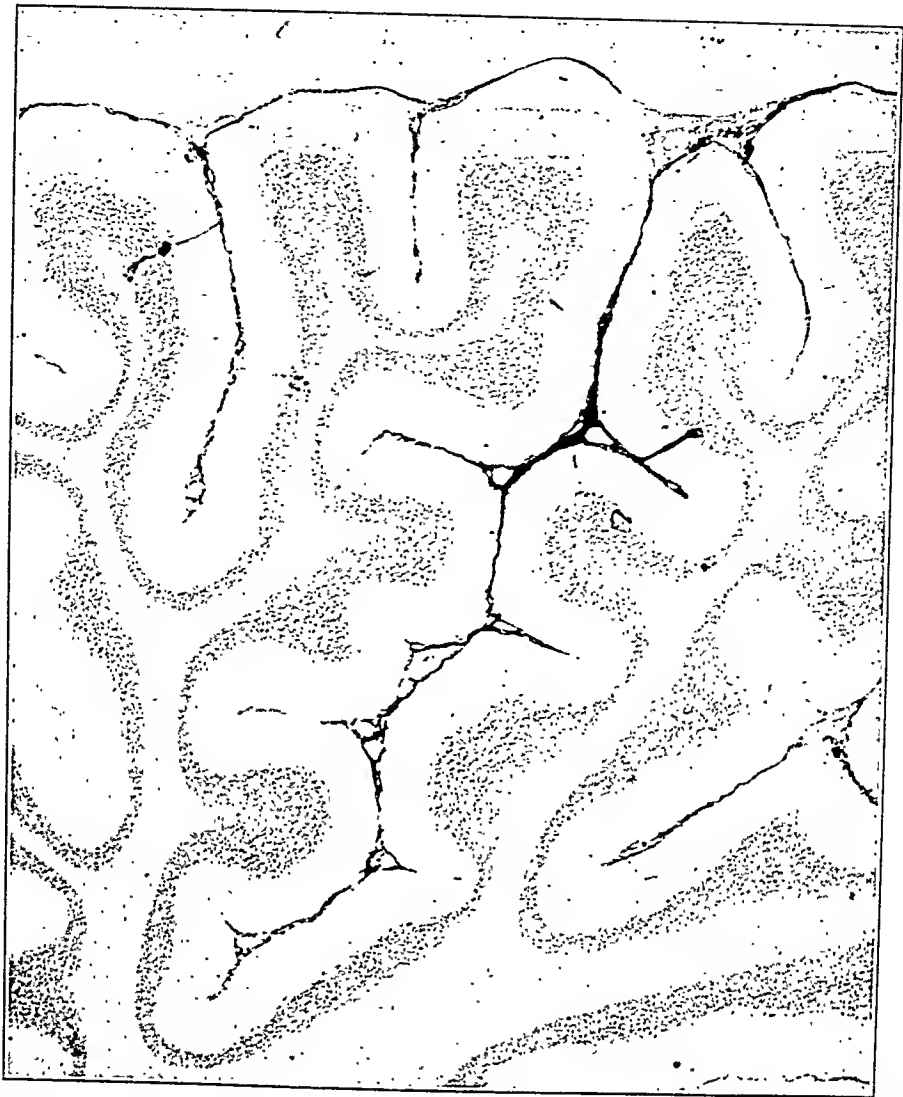
Primary Melanosarcoma of the Leptomeninges

PLATE 76

FIG. 2. The pia is diffusely involved by the melanotic process. The blood vessels entering the cortex are outlined by the melanin cells. These are within the perivascular spaces. Note the lack of pigmentation in the medullary layer. Thionin stain. $\times 15$.

FIG. 3. Marked infiltration of the meninges with pigment tumor cells. The invasion of the parenchyma by way of the perivascular spaces is well shown. In the cortex the perivascular spaces are filled with pigment cells. Van Gieson's stain. $\times 50$.

FIG. 4. Strip of pia showing the relation of the pigmented cells to the blood vessels. Thionin stain. $\times 100$.



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Primary Melanosarcoma of the Leptomeninges

PLATE 77

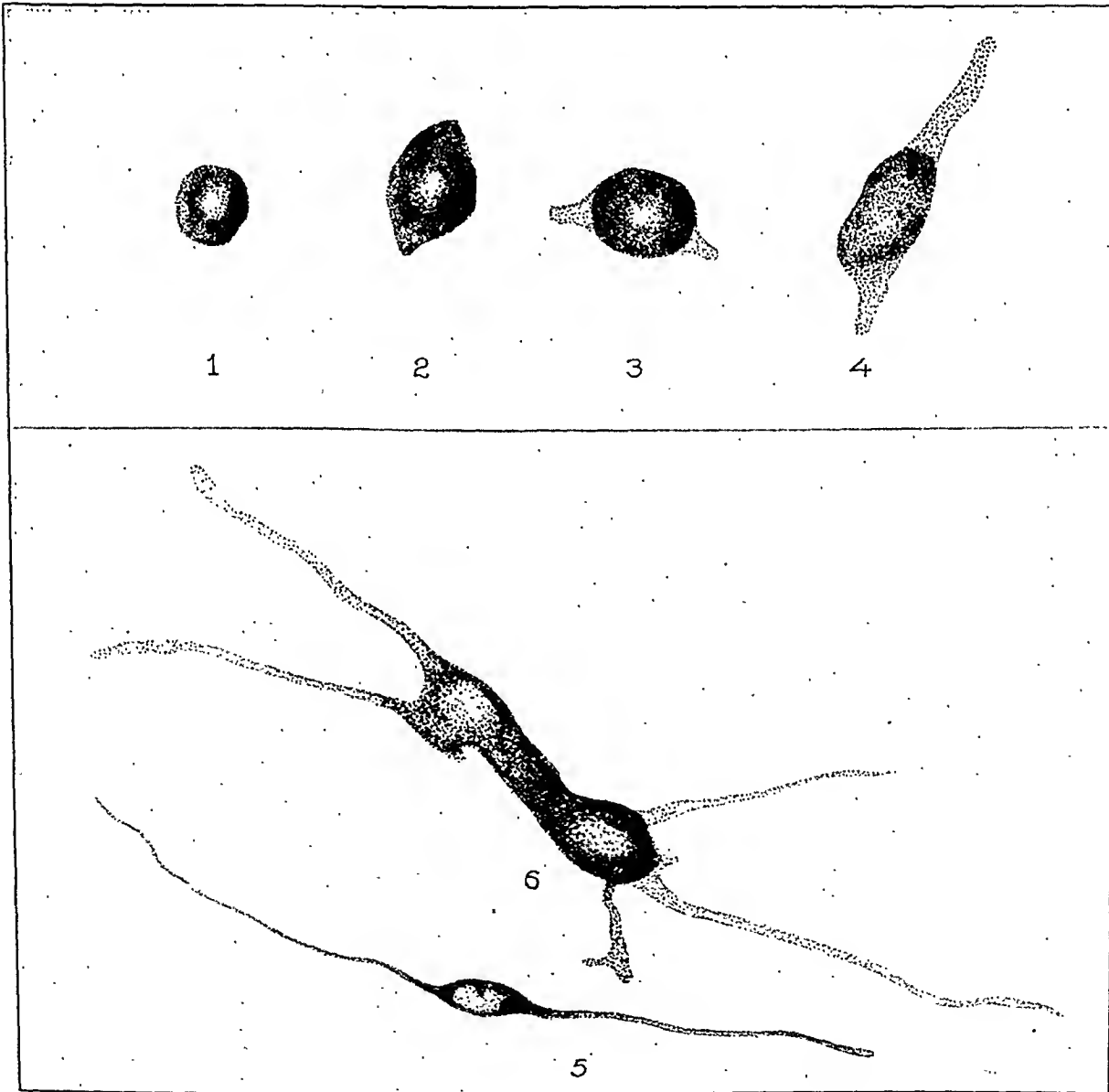
- FIG. 5. Small pigmented flecks in the pia. The chromatophores are seen radiating out from the center. Snessarew's modification of Cajal's technique. $\times 75$.
- FIG. 6. Drawing to show the various pigmented cells which form the pigmented fleck. Van Gieson's stain. $\times 75$.
- FIG. 7. Drawing to illustrate the development of the spindle-shaped chromatophore. 1 = round pigmented cell; 2 = oval pigmented cell without processes; 3 = oval pigmented cell with short processes; 4 = young spindle-shaped chromatophore; 5 = mature chromatophore; 6 = giant chromatophore with double nuclei. Unstained. $\times 300$.



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PLATE 78

FIG. 8. Drawing illustrating the development of the stellate chromatophore. 1 = round cell; 2 = polyhedral cell without processes; 3 = polyhedral cell with short processes; 4 = the mature chromatophore; 5 = chromatophore with double nuclei. Unstained. $\times 430$.

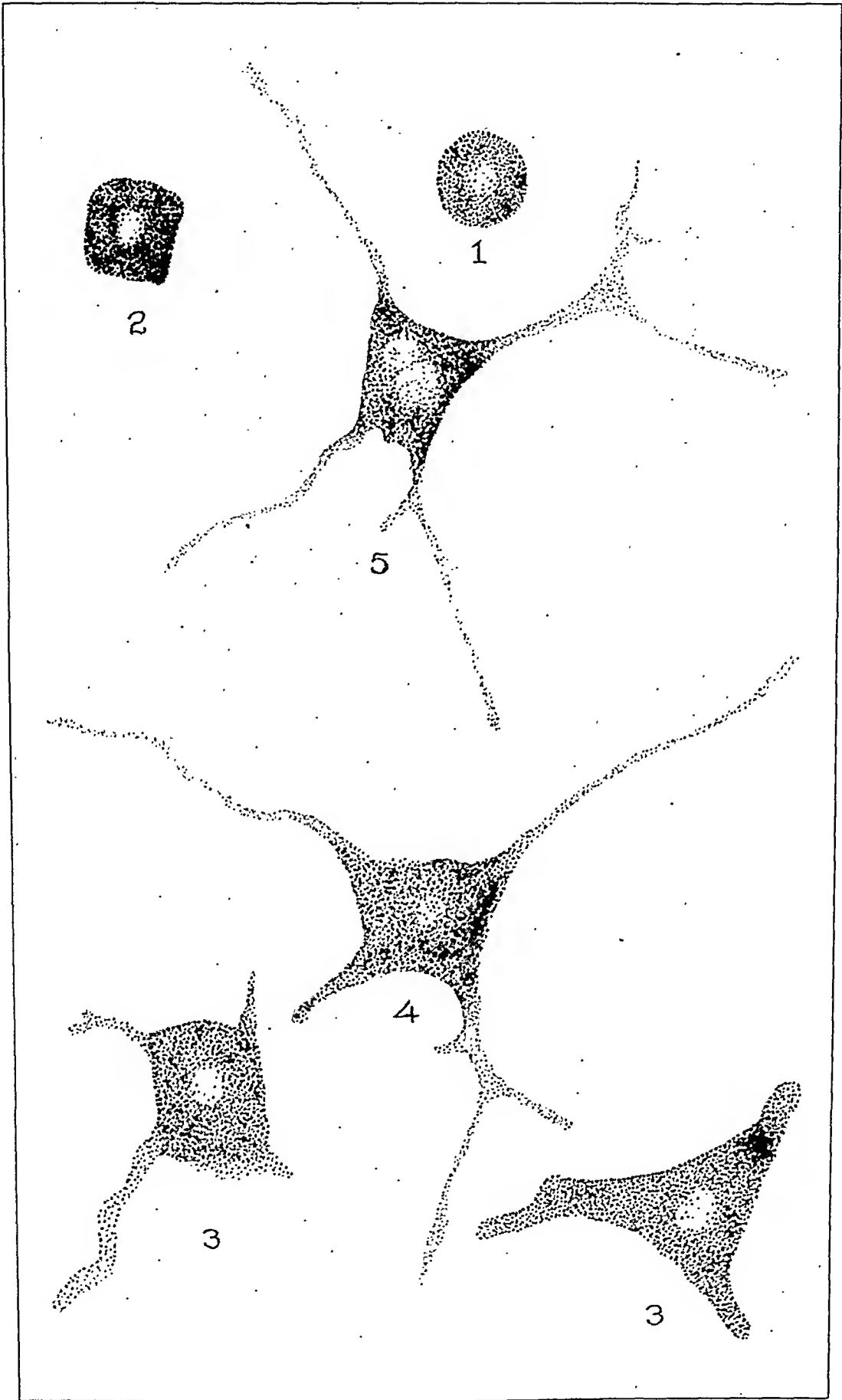
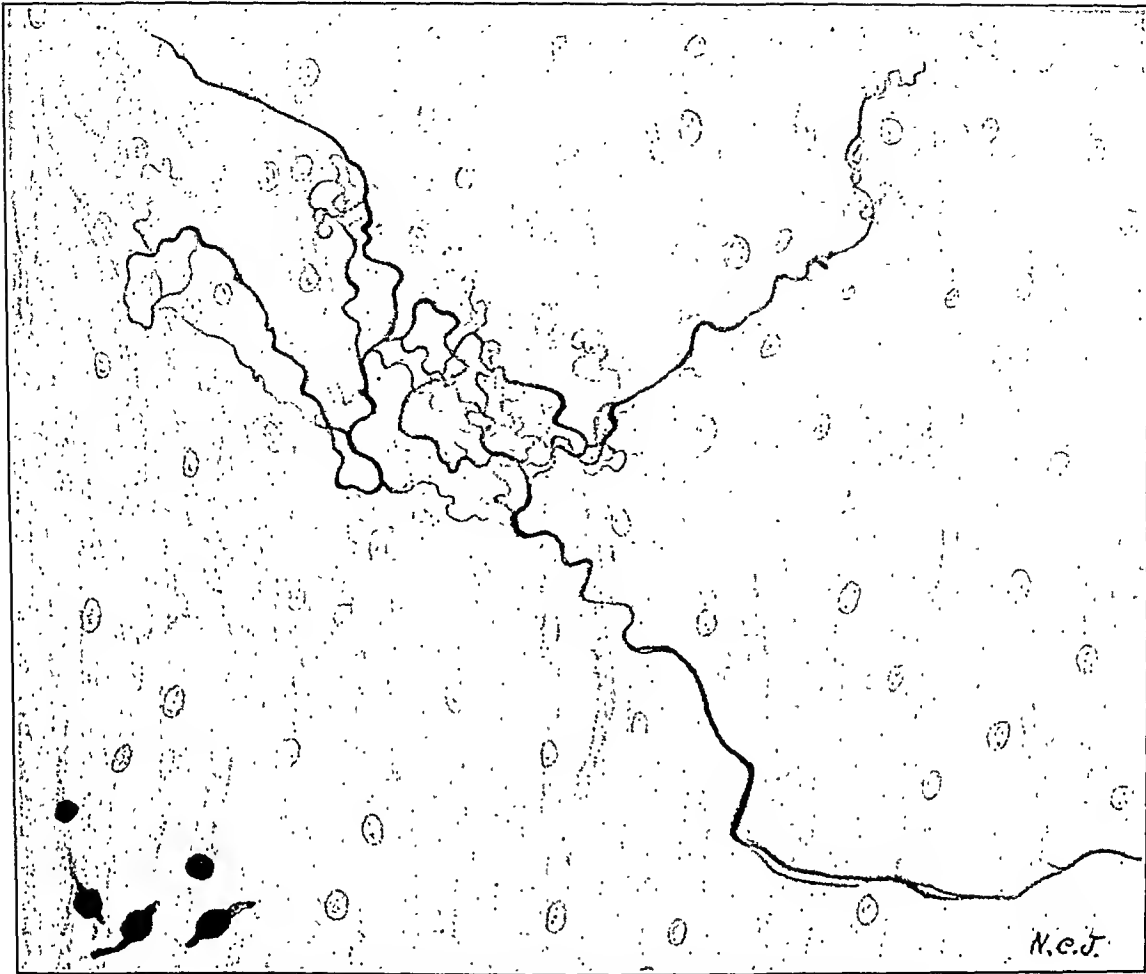


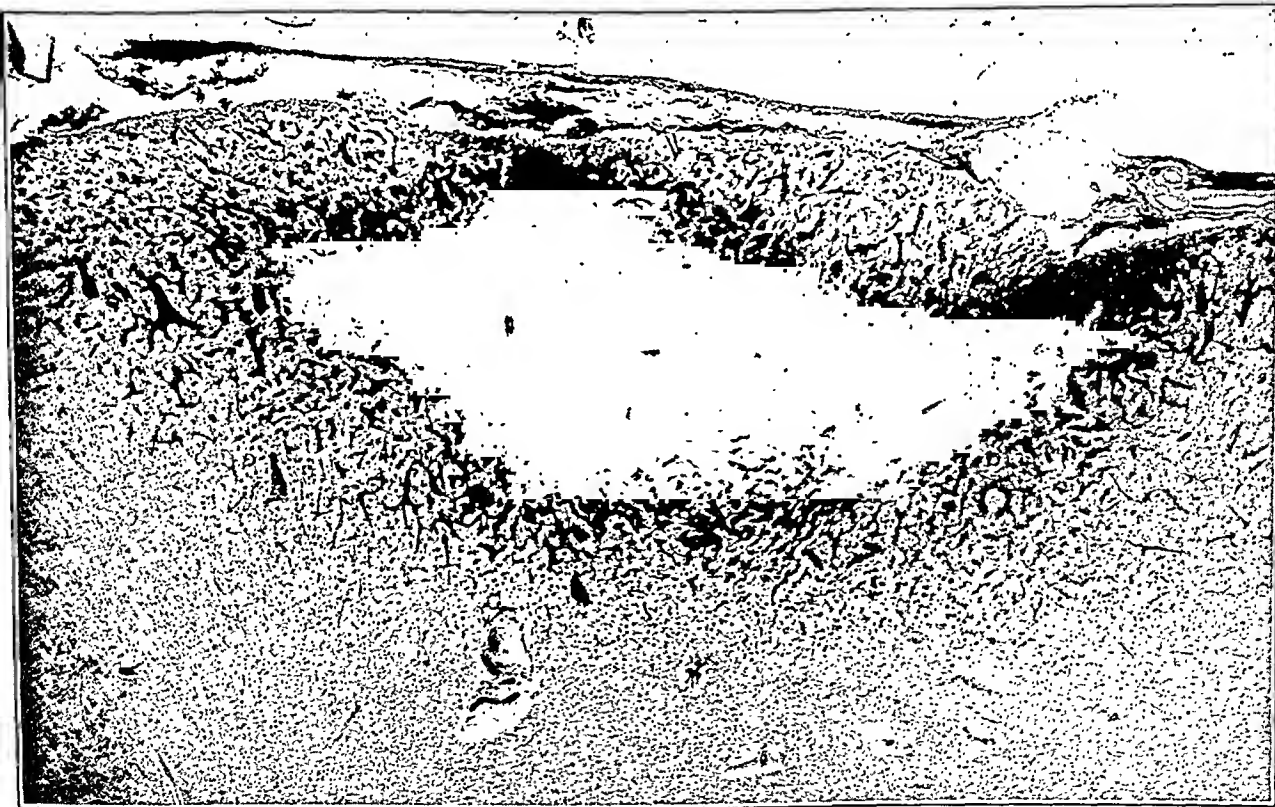
PLATE 79

FIG. 9. Drawing showing a nerve ending in the pia. Snessarew's modification of Cajal's technique. $\times 200$.

FIG. 10. Tumor nodule in the brain parenchyma. The infiltrative nature of the tumor into the surrounding brain substance is well shown at the periphery of the tumor. This infiltration occurs through the perivascular spaces and is limited to the cortex. The meninges show moderate infiltration. The dilated blood vessels are outlined by collars of pigmented cells. Hematoxylin-eosin stain. $\times 20$.



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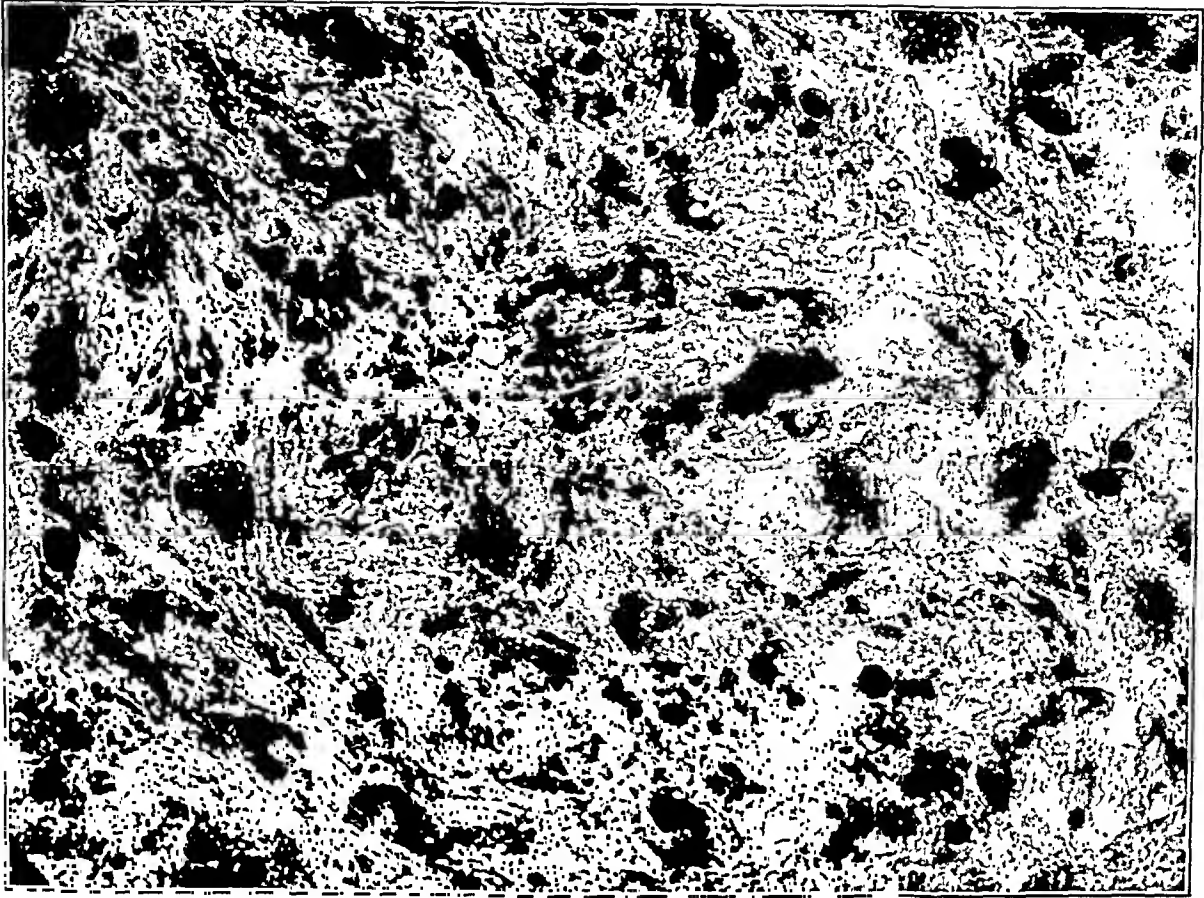


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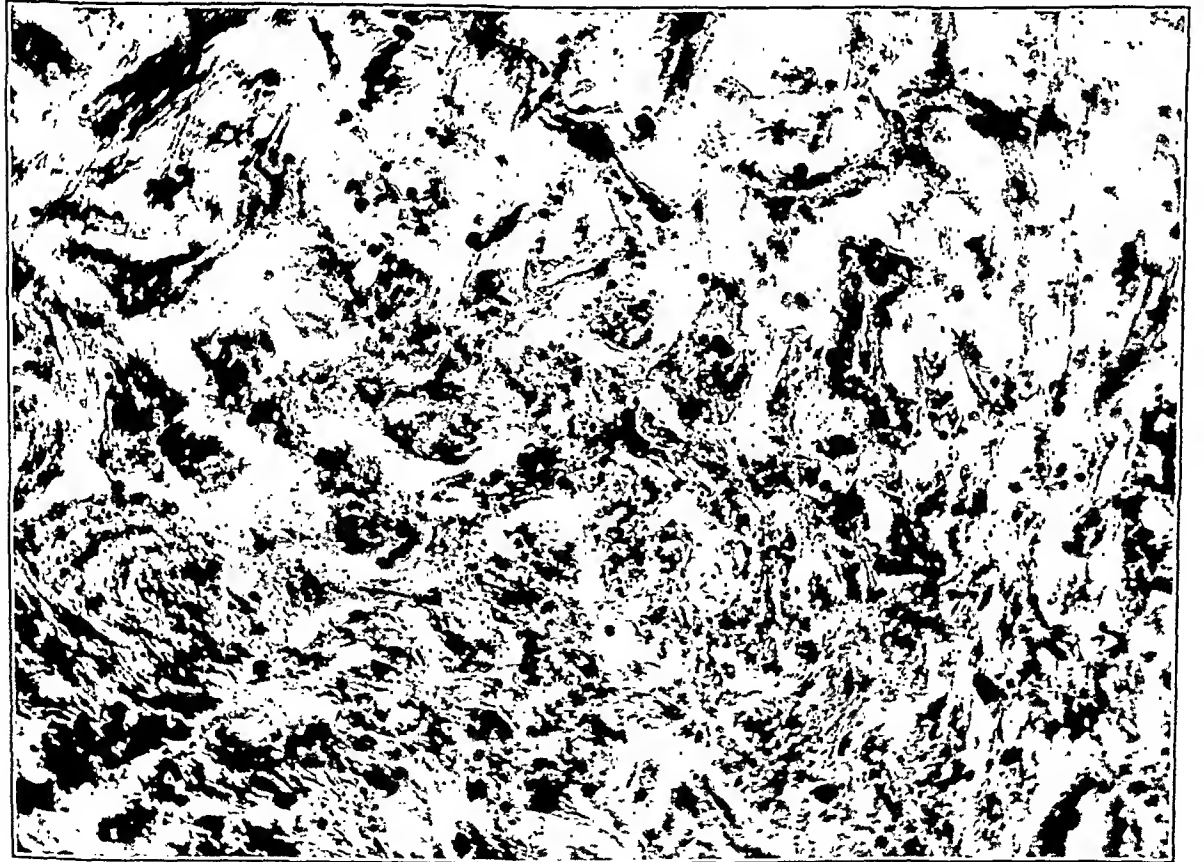
PLATE 80

FIG. 11. Field in the periphery of the tumor nodule showing the whorl-like formation of the tumor composed of spindle-shaped chromatophores. The perivascular spaces are filled with various types of pigmented tumor cells. Hematoxylin-eosin. $\times 100$.

FIG. 12. Interior of tumor in the cortex. The sarcomatous appearance is due to the compact arrangement of the spindle-shaped chromatophores. Numerous round and polyhedral pigmented cells are scattered throughout. Van Gieson's stain. $\times 430$.



I 2



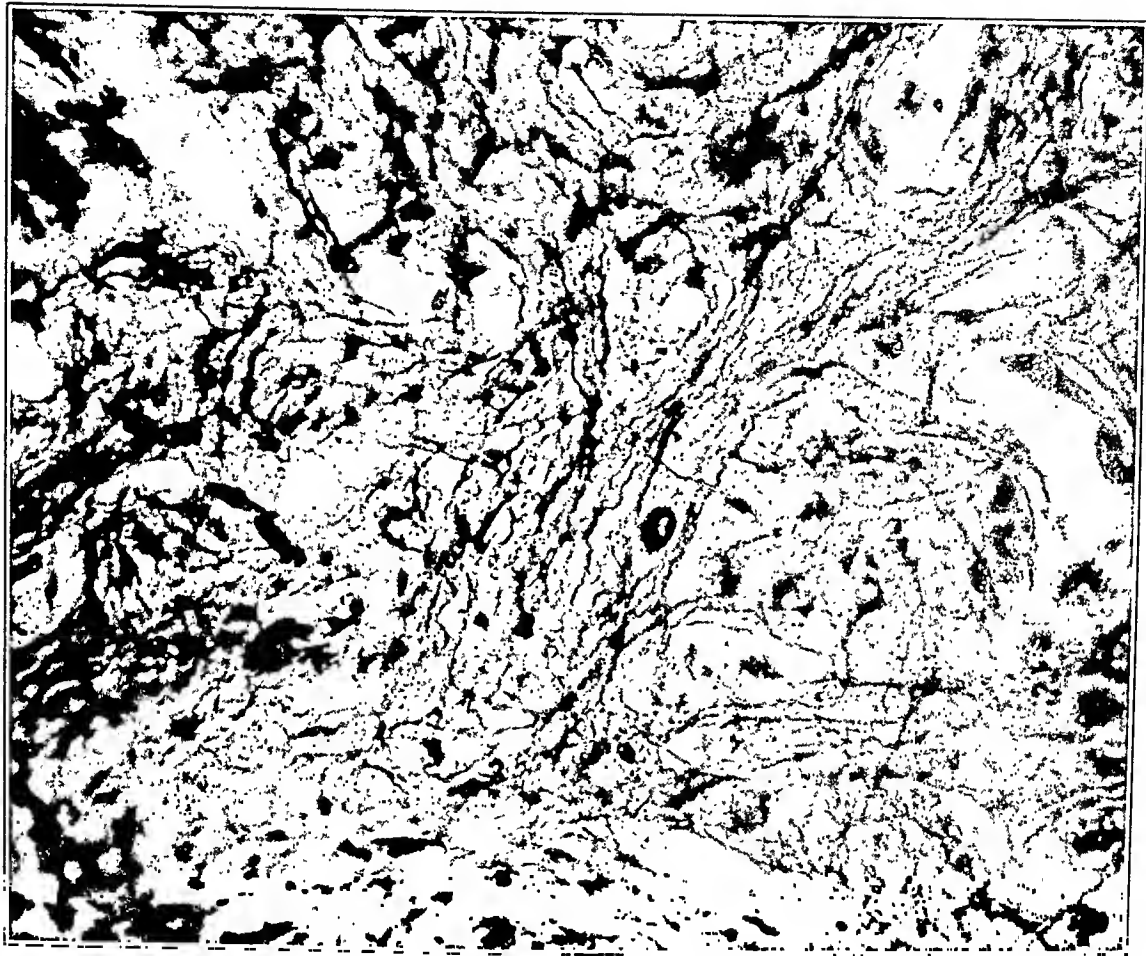
II

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Primary Melanosarcoma of the Leptomeninges

PLATE 81

FIG. 13. Field near the periphery of the tumor in the parenchyma showing reticulum and collagen fibers. The melanin granules are greatly decolorized. Perdrau's stain. $\times 430$.



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Primary Melanosarcoma of the Leptomeninges

THE INFLUENCE OF ANAPHYLACTIC SHOCK ON THE FINER STRUCTURE OF THE LIVER IN THE DOG *

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The symptoms of anaphylactic shock in the dog have been described so often as to become classical. Much is known, also, of the various physiological manifestations of anaphylaxis. But the histological descriptions are few, meager, and usually incidental to physiological or immunological studies. There is no adequate record of the finer histological or cytological details. It is my intention to describe some of the finer changes in the liver of the dog in anaphylactic shock and to attempt, at least, to correlate some of these changes with the already known physiological facts.

MATERIAL AND METHODS

Medium sized dogs (7 to 12 kg. body weight) were used for the experiments. Care was exercised in their selection, irritable and nervous animals being discarded.

White of egg, diluted 1:10 with distilled water and filtered, was injected subcutaneously in 5 cc. doses on 3 successive days, and after a sensitization period of about 3 weeks the dogs were prepared for experimentation. Food was removed in the late afternoon to insure that the cellular pictures of the liver would be approximately the same in all dogs. The following morning a long glass cannula was inserted, under local anesthesia, into the thoracic duct. The rise of lymph in the cannula was measured for a period of an hour. Then 20 cc. of the diluted eggwhite was injected intravenously. Almost immediately the dogs began to show symptoms of uneasiness and quickly went into shock. Individual dogs act differently, but usually there is a period of primary shock followed by signs of improvement or recovery. Generally, however, the period of improvement is of short duration, and is followed by a secondary shock, more severe and lasting longer than the primary one.

* The experimental part of this study was done in the Pathological Laboratory of the University of Illinois, College of Medicine, Chicago. I desire to thank Professor William F. Petersen for the facilities which were afforded me.

Received for publication March 15, 1935.

At different times, usually when the visible effects of shock had begun to cease, hepatic tissue was removed for microscopical study. An opening into the abdominal cavity was made under local anesthesia and in most cases a cannula was inserted into one of the large tributaries of the portal vein, instead of directly, so as to cause no interruption of the hepatic circulation. Sodium chloride in 0.9 per cent aqueous solution or a 10 per cent aqueous solution of sucrose at 38° C. was perfused through the liver and allowed to escape through an opening in the inferior vena cava above the diaphragm. Precaution was taken not to permit the pressure of the perfusing fluid to rise appreciably above the normal blood pressure of the dog. When the perfusing fluid began to escape only slightly tinged with blood the dog was killed with ether. The perfusion was continued for a short time, and then was followed by a warm mixture of 80 volumes of a 3 per cent aqueous solution of potassium dichromate and 20 volumes of formaldehyde solution. After some quantity of the fixation fluid had passed through the liver, the inferior vena cava and the portal vein were closed by ligatures. The liver was bathed *in situ* with the fixation solution, and in a half to 1 hour it was removed and small pieces cut out and put into fresh solution for 3 days. The pieces were then placed in an aqueous solution of 3 per cent potassium dichromate which was changed on alternate days for a week. In other dogs the livers were not perfused, but small pieces were cut out and fixed immediately. Material from the livers of normal dogs was treated in a manner similar to that from dogs in anaphylactic shock.

Serial sections were cut 5 μ thick from material embedded by the celloidin-paraffin method. The stains employed were Heidenhain's iron-hematoxylin, Mallory's phosphotungstic acid hematoxylin and Volkonsky's modification of the Altmann anilin - acid fuchsin method. Satisfactory and comparable results were obtained with all three stains. In addition some sections were stained with hematoxylin and eosin and by the Heidenhain "azan" method.

OBSERVATIONS

The Liver in Primary Anaphylactic Shock: The injection of antigen into a sensitized dog is followed quickly by an acute congestion of the liver and a marked fall in arterial blood pressure. The histological picture is one of passive hyperemia. In livers fixed within

15 minutes after injection, the sinusoids and central veins of the hepatic lobules are dilated widely. The sublobular and larger tributaries of the hepatic veins are found wide and patent. Less dilatation is observed in the branches of the portal vein. Beneath the capsule of Glisson and in the loose connective tissue of the portal spaces, also around the central veins, there occur small patches of hemorrhage.

Generally the endothelial lining of the hepatic sinusoids shows no morphological alterations. In places, however, the endothelium is thrust away from the parenchyma, and fine reticular fibrils bridge the space. Some polymorphonuclear neutrophile leukocytes and a few free histiocytes (reticulo-endothelial cells) are present in the sinusoids, besides in and around the central veins. A few erythrocytes may be seen between the sinusoidal endothelium and the hepatic parenchyma.

Concomitant with the passive hyperemia of the liver there occurs a marked increase of lymph-flow from the cannula inserted into the thoracic duct. Direct measurements of the lymph-flow reveal as much as an 8.5 times increase in the hour following the injection of antigen over the hour preceding. This lymphagogue effect begins immediately upon injection and reaches a maximum in 7 to 15 minutes, then diminishes, but the pre-injection level is not reached before 1 or more hours. There is an accumulation of lymph between the sinusoidal endothelium and the hepatic parenchyma: the lymphatic vessels in the walls of the central veins and larger tributaries of the hepatic veins are dilated widely, and considerable dilatation of the lymphatic vessels in the portal spaces occurs.

These early stages of primary anaphylactic shock display little parenchymatous damage. The hepatic cells in the central zone of the lobules reveal some swelling, their cytoplasm shows more granulations, and there is the beginning of "cloudy swelling." Cells adjacent to the central vein often show vacuolization of the cytoplasm. Karyorrhexis, or even loss of nucleus, was observed in a few instances.

Parenchymatous changes are not of the same degree in all livers. Individual dogs react differently, but the sequence of hepatic alterations is very similar in all animals.

The Liver in Secondary Anaphylactic Shock: Generally after 10 or 15 minutes, although the time may be prolonged a little, the dog in

shock begins to present a second series of symptoms. These symptoms are usually more severe than those of primary shock.

Marked swelling of the parenchyma is a feature of this stage. Considerable differences, however, are noted in individual hepatic cells. A large number of cells in the central zone of the lobule exhibit varying degrees of swelling, some being greatly swollen, while others show swelling to a lesser degree or not at all. In severe shock the swelling may extend into the midzone, but not even in dogs dying in shock was any disorganization observed in the peripheral zone of the lobule.

The swollen parenchymatous cells undergo typical "cloudy swelling": the cytoplasm shows more apparent granulation. The granules through imbibition become enlarged into minute vesicles, the centers exhibiting diminished reaction to acid dyes, while the peripheries stain well. A later coalescence of vesicles leads to vacuole formation. Most cells in the central zone of the lobule, and a large number of those especially in the first part of the midzone, appear vacuolated. In certain cells the vacuoles reach some size, leaving but a small area of cytoplasm around the nucleus and thin septa between the vacuoles. As a sequel to "cloudy swelling" hydropic necrosis sets in. Large numbers of cells in the central zone and inner parts of the midzone display vesicles containing the hyaline balls from hydrops, which stain well with eosin and Mallory's phosphotungstic acid hematoxylin.

Accompanying these cytoplasmic changes, the nuclei of some of the parenchymatous cells undergo progressive alterations. The changes are confined mainly to a few rows of cells surrounding the central veins. First the nuclei stain less well, then they become shattered, and finally there is a complete dissolution of the nuclear remnants.

The extensive swelling of the hepatic parenchyma leads to a narrowing of the sinusoids. Practically all of the sinusoids in the central zone of the lobule are clamped down, and there is a diminution in caliber usually of those in the midzone. The central veins and the tributaries of the hepatic veins, likewise, are diminished in caliber. Near the periphery of the lobule the sinusoids are considerably wider. In the narrowed sinusoids, some of which are so reduced that erythrocytes can pass through only in single file, there is a stasis of blood. Later the individual erythrocytes lose their identity. The

central sinusoids thereby are plugged by hyaline masses. Often the same is true of the much reduced central veins.

The endothelial lining of the sinusoids is pushed away from the hepatic cords, so that the Kupffer cells and endothelial cells stand out prominently. The reticular fibers of the Kupffer cells are clearly discernible. Sometimes erythrocytes and polymorphonuclear neutrophile leukocytes lie between the endothelium and the parenchyma. Occasionally the endothelial lining is broken. In places the endothelium seems thickened; whether the thickening is real or merely a deceptive appearance cannot be said. Near the periphery of the lobule, where the sinusoids are considerably wider, the endothelium adheres more intimately to the hepatic cells.

Within the sinusoids, also in the loose engorged connective tissue around the central veins and tributaries of the hepatic veins, are found numerous polymorphonuclear neutrophile leukocytes, a considerable number of free histiocytes, and a few lymphocytes. Cells of all these types are more numerous than during primary shock. The Kupffer cells and the histiocytes display considerable vacuolization. Some of the vacuoles contain a yellowish brown pigment or whole erythrocytes and those in various stages of disintegration.

Many of the hepatic cells in the centers of the lobules have taken up whole erythrocytes. An individual hepatic cell may contain from one to (by actual count) thirty erythrocytes within its body. This taking up of erythrocytes leads to much enlargement of the cells, the nuclei being pushed eccentrically. The number of hepatic cells taking up erythrocytes is proportional to the severity of the shock.

Preparations subjected to the Prussian blue reaction reveal a considerable amount of iron in some Kupffer cells, histiocytes and hepatic cells. The liver of the normal dog gave but a slight reaction. This increased reaction for iron becomes significant when the ingestion of erythrocytes and their subsequent disintegration are taken into account. But it must be borne in mind that the increased reaction for iron does not mean necessarily that all the iron has been derived from erythrocytes. Anaphylactic shock may have unmasked iron which is normally present and which is not displayed ordinarily.

An interesting feature of the dog's liver is the remarkable development of smooth musculature around the tributaries of the hepatic veins. The sublobular veins are surrounded more or less completely

by a thick layer of muscle, while the larger hepatic tributaries display more muscle tissue on one side than the other.

Chondriosomal Changes: Since chondriosomes are the easiest and earliest altered of the cytoplasmic inclusions, changes in these organoids may be expected in anaphylactic shock. Morphological descriptions of chondriosomes are beset with difficulties because of differences in functional conditions and in methods of technique. Nevertheless, certain changes in chondriosomes may be seen under both physiological and pathological conditions when the tissues are compared with those of normal animals kept under identical circumstances.

The hepatic lobule of the dog may be clearly marked out by the morphology of chondriosomes into three characteristic zones, although some differences are shown within each zone. In the liver of the normal dog chondriosomes are present in the parenchymatous cells in the form of spherules, rods and filaments. Suitably fixed and stained preparations show an abundance and fairly even distribution of chondriosomes. In dogs without food for 18 to 20 hours the hepatic cells in the central zone of the lobule contain a predominance of spherules, variable in size, although short rods are often present, while in individual cells filaments may be seen lying between the spherules and rods. Generally the cells, three or four deep, surrounding the central vein contain almost exclusively spherical chondriosomes or mitochondria. The chondriosomes in the midzone of the lobule are for the most part longer than in the central zone. Filaments abound, but some rods of different lengths and spherules are to be seen in many cells. In the peripheral zone of the lobule filaments — usually longer and thinner — appear in abundance. Individual cells, however, may contain a large number of rods and spherules. This is especially true of the most peripheral layer of hepatic cells adjacent to the portal spaces, where often are present spherules of greater diameter than found elsewhere in the lobule. Also, certain lobules have been observed in which cells abutting the portal spaces contain exclusively filamentous chondriosomes. The lability of these organoids makes it difficult to state just what is a normal appearance.

During the different stages of anaphylactic shock, and corresponding with the various degrees of shock, many alterations of chondriosomes occur in the hepatic cells. In mild or in primary

shock many of the spherical chondriosomes in the central zone of the lobule become swollen and vacuolated. Individual chondriosomes display clear centers and peripheral stained hulls. Sometimes the stained part is all on one side like a crescent. Diminution of staining capacity is very evident, and in some of the most central cells there is a complete loss of mitochondria by chondriolysis. In the midzone and peripheral zone of the lobule there is little or no change in the chondriosomes, except in certain cells of the midzone where they appear as swollen spherules and rods.

Severe anaphylactic shock causes greater alteration of the chondriosomes. In the hepatic cells surrounding the central vein of the lobules there may be a complete loss of mitochondria, depending upon the extent of the central necrosis. Further out, accompanying the "cloudy swelling," the changes in the chondriosomes go hand in hand with the general swelling of the granules of the ground cytoplasm and the vacuolization of the cells. Rods increase in caliber and segment into spherules; these swell, vacuolate, and some undergo dissolution. Cells filled with numerous or large vacuoles show a dispersion of chondriosomes between the vacuoles, around the nuclear membrane, and toward the periphery of the cells. In the midzone, particularly in the inner part, the filamentous chondriosomes are segmented mostly into rods and spherules. As a rule the degeneration of chondriosomes does not extend very far peripherally. Chondriosomes in the outer part of the midzone and in the peripheral zone, except in certain "dark cells," appear quite similar to those in the hepatic cells of normal dogs.

It is interesting to follow in some detail the sequence of degeneration of chondriosomes. Filaments (chondriocontes) may become beaded along their whole length, or in the middle, or may present blebs at one or both ends. Segmentation may occur between the beads, or the beads may swell and burst, thus shortening the filaments. A similar process occurs in the rods. Spherules (mitochondria) degenerate, first by swelling and vacuolization, followed by shattering and dissolution of the fragments. Chondriolysis, as here described, is usually an accompaniment to karyorrhexis and karyolysis. Another form of degeneration of chondriosomes will be recounted in connection with certain "dark cells."

Dark Cells: The literature abounds with descriptions of "dark" and "light" cells in the liver. In the liver of the normal dog both

dark and light cells are found in varying numbers. At the periphery of the hepatic lobule a single row, occasionally in places two cells deep, of dark cells lies tangential to the portal spaces and sometimes extends out along the interlobular vessels. In addition single dark cells or small groups of two, three, or four cells may be present almost anywhere in the lobule, especially in the midzone and peripheral zone. Anaphylactic shock appears to cause an increase of dark cells in both of these situations.

The peripheral or tangential dark cells are to be distinguished from the hepatic cells further in the lobule by their tinctorial reactions and by the character of their non-vacuolated, more or less homogeneous cytoplasm. In ordinary hematoxylin and eosin preparations the cytoplasm displays an intense affinity for the acid dye, besides often showing a distinct basophilic tinge. Mallory's phosphotungstic acid hematoxylin and Heidenhain's iron hematoxylin reveal an abundance of chondriosomes in the peripheral dark cells, so they may be spoken of as *chondriosome-rich* cells. From their location in the lobule, from their size and shape, and from nuclear and cytoplasmic characteristics these cells are without doubt hepatic cells. No transitional forms have been observed between them and the epithelial cells lining the intrahepatic bile ducts. Since the branches of the hepatic artery enter at the periphery of the lobule, it is reasonable to assume that these peripheral or tangential dark cells are better nourished than the cells further in. This increased nourishment may account, in part, for the abundance of chondriosomes. In many preparations the chondriosomes are present in the form of long slender filaments, but frequently the filaments are replaced by spherules, approximately twice the diameter of those in the hepatic cells around the central vein. The possible significance of these large spherules will be taken up later in the discussion. There can be scarcely any doubt that the peripheral dark cells are normal, because they display neither cytoplasmic nor nuclear signs of degeneration. Mitoses have been encountered in the peripheral dark cells, also cells have been observed with two nuclei.

The other more scattered form of dark cells, on the contrary, shows signs of both cytoplasmic and nuclear degeneration. The extensive destruction of the hepatic parenchyma in the center of the lobule incident to anaphylactic shock leads to a proliferation of cells further out, as attested both by mitotic figures and by the increment

of binucleate cells. Other cells, especially in the midzone and peripheral zone of the hepatic lobule, being perhaps toward the end of their cytomorphosis, undergo degeneration. These last cells display an enlargement of chondriosomes, which later become clumped together so that individual forms are not discerned, or are seen with difficulty. Accompanying the changes in cytoplasmic inclusions the nuclei undergo pyknosis. The cells become shrunken, irregular, and both nuclei and cytoplasm stain uniformly dark. Finally, only dark staining masses lie amid cells of perfectly normal appearance. These degeneration cells have been observed also on occasion lying between the undegenerated peripheral dark cells.

DISCUSSION

It is well established that anaphylactic shock in the dog is associated with marked circulatory disturbances which presumably are located on the venous side. The passive congestion of the intestines and liver, whether because of a relaxation of the capillaries which increases the capacity of these organs, or from some impediment to the outflow from the liver, causes an insufficient return of blood to the heart. Coincident with this passive congestion there is a marked fall in arterial blood pressure.

Manwaring¹ pointed out the importance of the liver in the production of anaphylactic shock in the dog. Voegtlin and Bernheim² and Denecke³ supported Manwaring's view, because when the liver was isolated from the circulation by an Eck fistula and ligature of the hepatic artery, it was impossible to produce the anaphylactic state. Subsequent investigators, while recognizing the rôle of the liver, have not been in agreement as to the mechanism involved by which this organ participates.

It was thought by Manwaring¹ that vasodilator substances liberated by the hepatic parenchyma act upon the systemic blood vessels and bring about a reduction of blood pressure. Biedl and Kraus⁴ believed that toxic peptone-like bodies derived by proteolysis from the injected antigen into a sensitized dog caused the fall in blood pressure, through a paralysis of the vasoconstrictor nerve endings. Weil⁵ was against the idea of a circulating toxin and assumed that reactions taking place in the sensitized hepatic cells are responsible for anaphylactic shock in the dog. The congestion of the liver was considered secondary to irritation of the parenchyma.

On the contrary, Simonds⁶ attributed the passive congestion of the liver in anaphylactic and peptone shock in the dog to a local venous constriction, caused by a spasm of the extensively developed smooth musculature in the walls of the hepatic veins and their tributaries. Mautner and Pick⁷ had shown that the hepatic veins of dogs were constricted by histamine. The existence of these atypical veins in the liver of the dog is well established and their spasmodic closure would help explain some of the similarities of anaphylactic, peptone and histamine shocks. But several investigators have entered objections to the theory of Simonds. Manwaring and Brill⁸ were unable to demonstrate a veno-constriction when a mixture of epinephrin and barium chloride, ergotin, or Vaughan's protein split-product was perfused through the isolated liver of the dog. Manwaring and his associates⁹ considered that one of the important factors in anaphylactic shock in the dog was increased permeability of the sinusoidal endothelium. An explosive edema accompanied by swelling of the parenchymatous cells of the liver increases local tissue pressure sufficiently to cause passive constriction of the sinusoids and hepatic veins. A stasis of blood in the sinusoids and narrowed hepatic veins is brought about by the increase of viscosity through the loss of fluid. Leukocytic deposits may be a minor factor in increasing the resistance.

Petersen and his collaborators^{10, 11, 12} have taken the similar stand, that anaphylactic shock in the dog consists primarily of an endothelial shock. As the result of stimulation, or of irritation, the permeability of the endothelium is increased, especially in the splanchnic area. An enormous quantity of fluid is forced into the hepatic lymphatics, distending the liver, disorganizing the capillaries, and allowing the escape of blood corpuscles into the lymph. The specific protein to which the cells have been sensitized leaves the capillaries rapidly and comes in contact with the parenchymatous cells, resulting in a primary shock. The passive congestion produces more injury, giving rise to a secondary shock. An "endothelial blockade" accomplished by injections of saccharated oxide of iron alters the intensity of the shock, either as a true blockade, or by increasing the activity of the endothelium, involving perhaps a more rapid destruction of the antigen, thereby protecting the parenchymatous cells.

Finally, Zinsser¹³ in summary wrote: "While we have no positive

knowledge of the site of the anaphylactic reaction within the body, it is more than likely that the primary point of attack is in the reticulo-endothelial system." Even if this supposition be true, we do not know whether the antigen injected into a sensitized dog acts directly upon the endothelium, increasing its permeability, or upon nerve endings, affecting the vasomotor mechanism. A study of the possible influence of the nervous system in the production of anaphylactic shock is now in progress.

How is the remarkable increase of lymph-flow from the thoracic duct during anaphylactic shock to be accounted for? The well known conclusions of Starling¹⁴ that "the whole increase of lymph obtained on obstruction of the inferior vena cava above the diaphragm is derived from the liver," have not been substantiated fully by more recent investigators. Markowitz and Mann¹⁵ thought that the liver, while contributing a part of the lymph to the thoracic duct, played a lesser rôle than supposed by Starling. When the periportal lymphatics were ligated no change was noticed, except a temporary increase of lymph-flow, which could be attributed to the intestines and operative manipulations. After the removal of the liver, there was found no diminution of lymph-flow. Peptone produced the usual lymphagogue effect in the hepatectomized dog, in which Markowitz and Mann considered that the lymph came largely from the intestines. Drinker and Field¹⁶ are inclined, likewise, toward the idea that the liver is not, but the intestines are, the main source of lymph from the abdomen.

The increase of intraportal pressure, owing to the congestion of the liver and other organs of the splanchnic area, favors the passage of fluid out of the capillaries as lymph. Asphyxia is an early symptom of acute anaphylactic shock; this deprivation of oxygen to the tissues would increase capillary permeability and facilitate the flow of lymph. While we can argue only from analogy, the chemical studies of Petersen and his associates are convincing that anaphylactic shock in the dog alters the lymph picture and strongly suggests an increase of capillary permeability.

Serial sections of the liver of the dog reveal two sets of lymphatic vessels, — one in the portal spaces, the other around the tributaries of the hepatic veins, and none within the hepatic lobules. Similar observations have been pointed out by Herring and Simpson¹⁷ in the dog and cat, and by Lee¹⁸ who ligated the thoracic duct in the

cat and obtained a retrograde injection of the lymphatics in the liver. Since the liver has a dual drainage, part to the hepatic lymph glands and part to the diaphragmatic lymph glands, it seems reasonable that some lymph from the organ never reaches the thoracic duct, but flows eventually into the right lymphatic duct. For this reason, and because of the congestion of the intestines, in anaphylactic shock we have no idea how much of the increase of lymph-flow from the thoracic duct is derived from the liver.

The congestion of the sinusoids and efferent veins of the liver and the swelling of the parenchymatous cells in the centers of the hepatic lobules in primary anaphylactic shock confirm the observations of Weil,⁵ Manwaring, French and Brill,⁹ and Dean and Webb.¹⁹ It is only in secondary shock, where an increased swelling and necrosis of the cells is accompanied by perivascular edema, that a narrowing of the sinusoids, hepatic veins and their tributaries occurs. Mallory,²⁰ in introducing the term central necrosis, described a comparable picture of the liver, and thought that this type of necrosis was brought about by toxic action. Since the parenchymatous cells in the centers of the lobules are not so well nourished as those toward the periphery, being further removed from the blood brought in by the hepatic artery, they are more labile and susceptible to injury. Later observers, as Zimmerman and Hillsman,²¹ Bolton and Barnard,²² and Simonds and Callaway²³ agree with Mallory that the centers of the hepatic lobules are less resistant because they are removed further from the source of nutriment, but doubt that toxic action plays a rôle in the production of central necrosis from venous stagnation.

From the above accounts it would appear that uncomplicated venous stasis in the liver causes injuries to the parenchymatous cells, which parallel those brought about by anaphylactic shock. There occur the same parenchymatous swelling, karyorrhexis and karyolysis, followed by cytolysis of the cells in the central zone. The sinusoids are narrowed by the swelling of the parenchyma and the perivascular edema, and hyaline thrombi appear in many places. But the presence of focal necroses in the midzone and peripheral zone of the hepatic lobule, in which the cells undergo homogeneous atrophy, does not occur in cases of simple passive congestion of the liver. Such necroses were looked upon by Flexner,²⁴ Mallory,²⁰ Pearce,²⁵ Fiessinger,^{26, 27} and Karsner and Aub²⁸ as toxic in origin.

I am of the same opinion, and in addition regard the extensive lesions in the centers of the hepatic lobules in secondary shock as of toxic origin. This view is shared by Weil, Manwaring, Dean and Webb, and others from observations on anaphylactic shock in the dog, and by Apitz²⁹ in the rabbit. Furthermore, how are we to explain the clamping down of the hepatic veins and their tributaries on the ground of simple uncomplicated passive congestion? Here again it seems that there must be some toxic product liberated in anaphylactic shock which acts upon the extensively developed smooth musculature in these veins, since constriction was observed only in secondary shock. Finally, the taking up of whole erythrocytes by the hepatic cells, a feature of severe anaphylactic shock in the dog, is unknown as a sequel to passive hyperemia of the liver. Browicz^{30, 31} in a series of papers reported the presence of whole erythrocytes in the hepatic cells of the dog following intravenous injections of hemoglobin and of defibrinated blood. He accepted the existence of intracellular canaliculi communicating with the sinusoids, a view that has not remained unchallenged. Rössle,³² in a case of human hepatic cirrhosis, observed erythrocytes not only in the parenchymatous cells of the liver, but also in the pancreas and kidney. Capillary failure was attributed as the cause. The evidences of increase of capillary permeability, disorganization of the sinusoidal endothelium and parenchymatous necroses detailed in severe anaphylactic shock in the dog make it quite possible that erythrocytes may pass either by diapedesis through the endothelium or directly into the injured hepatic cells. The increment of iron seen by me, and described by others, in the Kupffer cells and parenchymatous cells is at least suggestive in this connection.

Mayer, Rathery, and Schaeffer³³ studied the degeneration of chondriosomes in the liver and emphasized the ease with which these cytoplasmic inclusions are altered. They pointed out two types of alterations, namely, by cytolysis and by homogeneous atrophy. Both of these types of chondriolysis were stated to occur after various poisons, mineral and organic, also as the result of microbic toxins and those of autolysis. Similar types of degeneration present themselves in the hepatic cells of the anaphylactic dog. In the first type the chondriosomes, particularly in the central zones of the hepatic lobules, undergo segmentation, round off and become transformed into vesicles with clear centers. A complete dissolution is

the final outcome. This type of chondriolysis manifests itself in the cells where "cloudy swelling" is most evident. Smith and Rettie³⁴ reported similar observations. It is probable that some of the hypertrophied granules mentioned by Landsteiner³⁵ in his study of "cloudy swelling" are likewise mitochondria. Hypotonic solutions according to Bang and Sjövall,³⁶ Anitschkow,³⁷ Smith and Rettie,³⁴ and others produce the same type of chondriosomal degeneration. It would appear that the imbibition of fluid from the ground cytoplasm causes the chondriosomes to swell, lose their staining capacity, and finally to undergo dissolution.

The homogeneous atrophy of chondriosomes was observed only in dark cells of the degeneration type. These cells, confined mostly to the midzone and peripheral zone of the hepatic lobule, are without doubt toward the end of their cytomorphosis. Fiessinger²⁷ included them in his general group of dark cells, for which he offered three interpretations: (1) artifact of preparation; (2) state of functioning; (3) pathological alteration. That the cells are artifacts he thought could be dismissed, because with the same fixation hepatic dark cells are found rarely in the guinea pig and frequently in the dog. Moreover, using mitochondrial methods, chondriosomes are brought out very clearly and evenly spaced in the light cells, whereas they are condensed and lie close together in the dark cells. Cohn³⁸ distinguished light and dark cells in the liver of the mouse, dog, cat, rabbit and guinea pig, and thought that the two appearances represented a different functional state. A number of authors from inanition studies have concluded the same thing, because in a state of complete inanition the number of dark cells is increased, while in well nourished animals their number is reduced or absent. I believe that this is true, as regards the peripheral dark cells, but the condition of the chondriosomes in the more scattered form is evidence to the contrary. The third interpretation, that the dark cells represent pathological alterations, has the support of the experimental studies of Fiessinger,²⁶ Policard,³⁹ Fiessinger and Lyon-Caen,⁴⁰ and several later workers. From the appearances that I have observed in the livers of dogs in different stages of anaphylactic shock, and shock of varying degrees of severity, I think that there are two types of dark cells; one type, clearly degenerative, may be derived either from light cells or from the other type, the peripheral or tangential dark cells. These peripheral or tangential dark cells, distinguished by

Rumjanzev,⁴¹ Kutsuna,⁴² Rabl,⁴³ Böhm,⁴⁴ Clara,⁴⁵ Pfuhl,⁴⁶ and others represent without much doubt normal cells. The chondriosomes in these dark cells, besides being very abundant, appear normal. The suggestion of Böhm⁴⁴ and of Pfuhl⁴⁶ that these cells represent functional states is timely, because the periphery of the hepatic lobule is better nourished, receiving blood from both branches of the hepatic artery and portal vein.

In certain cells or groups of cells on or near the periphery of the hepatic lobule, particularly adjacent to the portal spaces, large spherical chondriosomes occur having about twice the diameter of the spherules in the cells surrounding the central vein. Because of the location of these cells it was considered that a stasis of bile might account for the appearances of the chondriosomes. Fiessinger and Lyon-Caen⁴⁷ observed the transformation of rod-like chondriosomes into spherules when a hypersecretion of bile was provoked in the dog by intravenous injections of hemoglobin. Bang and Sjövall³⁶ described chondriosomes becoming spherical when pieces of frog's liver were treated with bile. Albot⁴⁸ noticed the same effect after ligation of the common bile duct in the rabbit.

SUMMARY

1. Anaphylactic shock in the dog presents two characteristic stages: (a) a primary shock, usually of short duration; and (b) a secondary shock, more severe and prolonged.

2. An extreme congestion of the liver, a marked fall in arterial blood pressure, and an increase of flow of lymph from the thoracic duct are displayed as features of primary shock. Some swelling of the hepatic cells appears in the centers of the lobules.

3. Secondary shock produces more damage to the parenchyma of the liver, as "cloudy swelling," hydrops, vacuolization of the cytoplasm, and finally a central necrosis. Accompanying these degenerative changes there is found a diminution in caliber of the sinusoids of the hepatic lobules, also of the efferent veins of the liver. A stasis of blood in the narrowed sinusoids leads to formation of hyaline plugs or thrombi.

4. A disorganization of the sinusoidal endothelium, proliferation of the endothelial cells, and increased phagocytosis by the Kupffer cells are very evident. The taking up of whole erythrocytes by the

parenchymatous cells of the liver is noted in severe secondary shock. Both the hepatic cells and Kupffer cells reveal an increment of iron.

5. Chondriosomes reflect the extent of parenchymatous injury. In the centers of the lobules spherical chondriosomes abound. Later, in some of the most central cells, chondriolysis occurs accompanying karyorrhexis and karyolysis. Homogeneous atrophy preceded by chondriomegaly appears in certain cells or groups of cells in the midzone and peripheral zone of the hepatic lobule. Two forms of "dark cells" may be differentiated on the basis of nuclear and cytoplasmic appearances.

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DESCRIPTION OF PLATES

PLATE 82

Figures 1 to 6 were drawn with the aid of a camera lucida at an approximate magnification of $360\times$ from preparations fixed in Regaud's potassium dichromate and formaldehyde mixture and stained by Mallory's phosphotungstic acid hematoxylin method.

FIG. 1. Normal dog. A sinusoid is shown entering into a central vein of the liver. Chondriosomes are pictured largely as spherules, while some rods and short filaments are present.

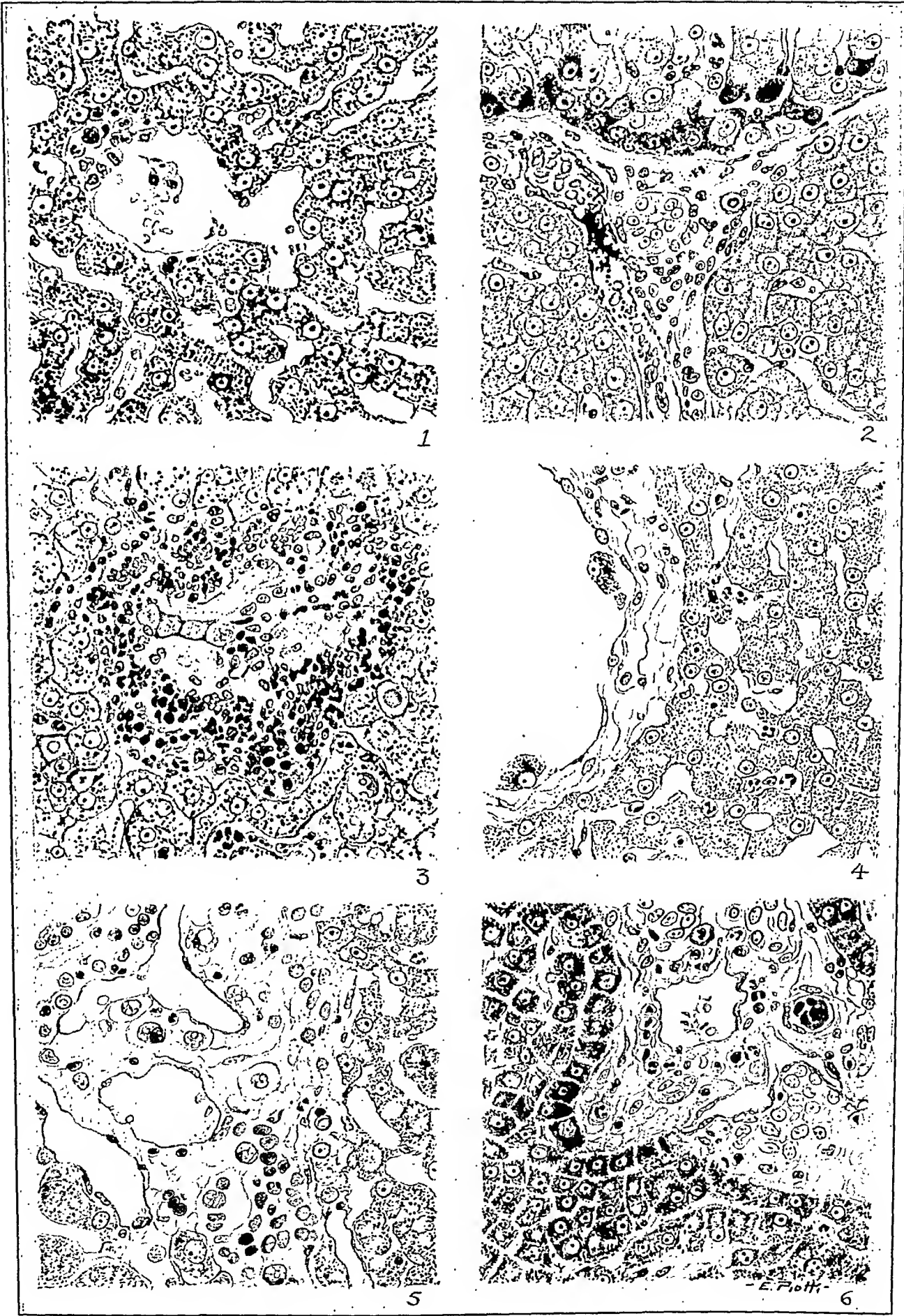
FIG. 2. Normal dog. Portal space. Tangential dark cells are shown at the periphery of a hepatic lobule.

FIG. 3. Anaphylactic dog. Secondary shock. A central vein is depicted filled with a hyaline mass and blood cells. Note the group of detached hepatic cells which lie within the lumen of the vein. Some hepatic cells appear swollen and contain hyaline balls of hydropic necrosis. The sinusoids show narrowing.

FIG. 4. Anaphylactic dog. Primary shock. The sinusoids are not narrowed. Pericentral lymphatics are distended. Free hepatic cells lie in the lumen of a central vein.

FIG. 5. Anaphylactic dog. Primary shock. Notice that the pericentral lymphatics display greater distention than those in Fig. 4. Erythrocytes may be observed within the lymphatic vessels. Histiocytes, leukocytes and erythrocytes are present in the connective tissue sheath of the vein.

FIG. 6. Anaphylactic dog. Secondary shock. Portal space. The lymphatic vessels show some dilatation. Tangential dark cells are portrayed mostly with filamentous and rod-like chondriosomes.



Weatherford

Influence of Anaphylactic Shock on Liver

PLATE 83

Figures 7, 9, and 11 were drawn at an approximate magnification of $940\times$ and Figures 8, 10, and 12 of $1150\times$ from preparations fixed in Regaud's potassium dichromate and formaldehyde mixture and stained by Mallory's phosphotungstic acid hematoxylin method.

FIG. 7. Anaphylactic dog. Secondary shock. Hepatic cells adjacent to the central vein show spherical and rod-like chondriosomes, with beading of some of the longer forms.

FIG. 8. Anaphylactic dog. Secondary shock. Central zone. Two hepatic cells display a taking up of erythrocytes.

FIG. 9. Anaphylactic dog. Secondary shock. Peripheral zone. "Dark" and "light" cells. Notice that there is no evidence of chondriolysis.

FIG. 10. Anaphylactic dog. Secondary shock. Midzone. Two cells show hydrops. Leukocytes abound in the sinusoids.

FIG. 11. Anaphylactic dog. Secondary shock. Section at the periphery of two lobules showing an intralobular bile duct cut longitudinally. Notice the abundance of chondriosomes present in the tangential dark cells.

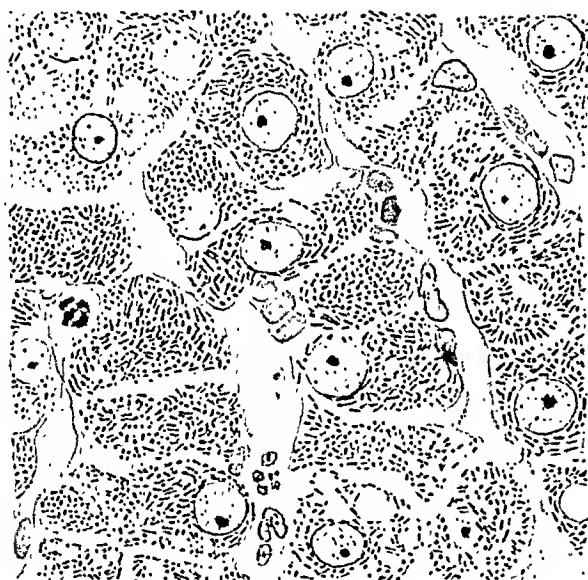
FIG. 12. Anaphylactic dog. Secondary shock. Periphery of a lobule. The chondriosomes in the dark cells display a more or less proximodistal arrangement.



7



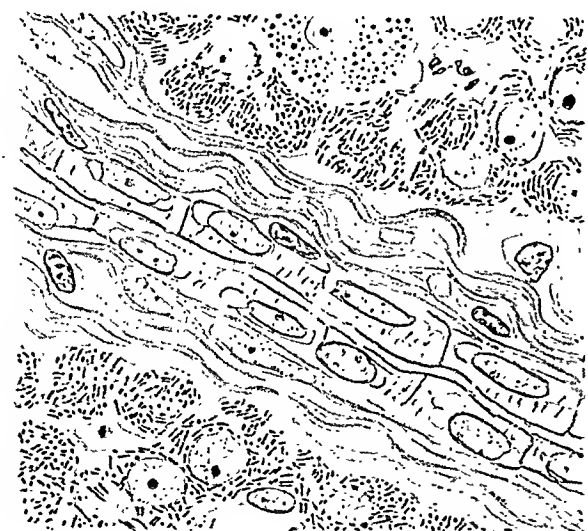
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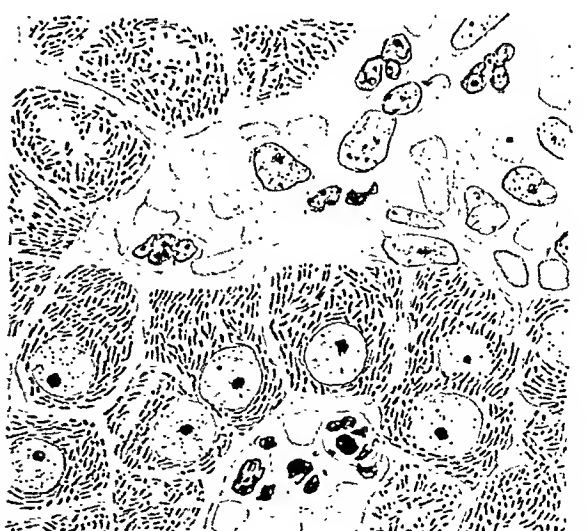
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- E. P. J. -

LESIONS IN THE ROOTS OF THE PULMONARY ARTERY AND AORTA IN RHEUMATIC FEVER *

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When blocks are cut from the heart according to the standardized technique suggested by Gross, Antopol and Sacks,¹ it becomes possible to study approximately 1.5 to 2 cm. of the aortic and pulmonic roots, *i.e.* the last musculo-elastic portions of the vessels prior to their transformation into connective tissue annuli. In the routine examination of a large number of active and inactive rheumatic hearts employing this standardized method, a surprisingly high incidence of pathological lesions was observed both in the roots of the pulmonary artery and aorta proper, as well as in the vessels within their enveloping pericardial mantles. The lesions occurring in the walls of the arch, and ascending and descending portions of the aorta † have been described in considerable detail by Klotz,³ Pappenheimer and VonGlahn,^{4, 5, 6} Chiari,⁷ Shaw,⁸ Giraldi,⁹ Perla and Deutch,¹⁰ McClenahan and Paul,¹¹ Gray and Aitken,¹² and Klinge.¹³ Those occurring within the walls of the pulmonary artery seem, for the most part, to have escaped notice. The only reports available on this subject appear to be those by Paul,¹⁴ who was able to substantiate Sacks' ¹⁵ prediction that such lesions would be found, and by Kugel and Epstein,¹⁶ Shaw,⁸ Gray and Aitken,¹² and Chiari.¹⁷ None of these reports, however, lays special emphasis on the roots of these vessels. Kugel and Epstein appear to be the first to have called attention to the frequency with which lesions may be found in the "musculo-arterial junction" in the acute cases. By this term they referred to the region where the annulus is inserted into the myocardium, an area that properly belongs to the valve ring. They indicated the possibility that the dilatation of the aortic ring, which frequently occurs in acute cases, may bear some relation to the damage found early in this area.

* Aided by grants from the Lucius N. Littauer and Walter W. Naumburg Funds.

† Inflammatory nodules in the root of the aorta in rheumatic fever were observed by Coombs in 1908.²

Received for publication January 9, 1935.

In this report it is proposed to describe the lesions found within the roots of the pulmonary artery and aorta proper, *i.e.* the purely musculo-elastic portions, as well as within the vessels found in their enveloping pericardial mantles. These lesions will be considered statistically with respect to their incidence in active and inactive rheumatic cases as well as in control material. A discussion will be given of their possible significance.

MATERIAL AND METHODS

The findings in 150 hearts form the basis of this report. Of these, 66 presented active rheumatic fever as manifested by the presence of fibrinous pericarditis, acute verrucous endocarditis, Aschoff bodies, eosinophilic collagen changes (fibrinoid) and other inflammatory phenomena in the myocardium. Thirty-four cases represented inactive rheumatic material, according to the criteria formulated by Rothschild, Kugel and Gross.¹⁸ The remaining 50 cases were from a non-rheumatic control series representing various age periods from birth to the ninth decade of life. This series was carefully selected to eliminate past or present hypertension since this condition is associated with vascular changes in the periaortic and peripulmonic sheaths.* Syphilis was excluded by the history, corroborative pathological findings or positive Wassermann test. After fixation of the heart in 10 per cent neutral formalin saline,† blocks were cut according to the standardized method of Gross, Antopol and Sacks. Sections were cut from each block 7.5 μ thick and stained as a routine with hematoxylin and eosin and with Weigert's elastic and Van Gieson's connective tissue stains.

ROOT OF THE PULMONARY ARTERY PROPER

In an examination of the non-rheumatic control hearts it was found that occasional capillaries were present between the medial fibroelastic and muscular strands of the pulmonary artery root in approximately 24 per cent of the cases. These capillaries are extremely inconspicuous and rarely penetrate inward beyond the outer third of the media. As compared to this, the root of the pulmonary artery

* The peripulmonic and periaortic sheaths mentioned in this report refer to the pericardial and adventitial mantles around the roots of these vessels.

† Solution of formaldehyde U. S. P., 10 parts; 1 per cent sodium chloride solution, 90 parts. This solution is rendered neutral with a weak alkali.

in the inactive rheumatic hearts presented capillaries in 70 per cent of the cases. These were not infrequently larger, somewhat irregular and penetrated at times into the middle third of the media, more rarely into the inner third (Fig. 1). There were no inflammatory cells seen surrounding these capillaries. At times, however, a scant amount of connective tissue was noted about them.

When this connective tissue around the capillaries becomes somewhat more conspicuous one may speak of "scarring" within the media. Medial scarring is usually associated with some change in the nature, amount and distribution of the elastic membranes. These may be absent, distorted, ruptured, frayed or even markedly increased. Moderate grades of scarring and alterations in the structure of the elastic membranes are difficult to discern in the root of the pulmonary artery because of the anatomical peculiarities of this site. Thus, in the normal pulmonary artery root there is a very considerable variation in the arrangement of the elastic lamellae, fibrous tissue and smooth muscle cells. In contrast to the histological structure of the aortic root, the region where the pulmonary artery proper passes into its annulus insertion often displays marked irregularities in the distribution and orientation of its elastic and muscular fibers which form an uneven mosaic with patches of fibrous connective tissue. The elastic fibers frequently take the form of spiral and circular arches. Because of these peculiarities in structure, cross-section of the pulmonary artery root is apt to present what appear to be discontinuities of the elastic membranes, and irregular areas of connective tissue devoid of smooth muscle or elastic fibers.

Another confusing element is the fact that the pericardial sheath surrounding the pulmonary artery root is generally in close apposition to it, especially during the early age periods. With increasing age, a looser fibro-adipose tissue makes its appearance. Within this pericardial mantle there are to be seen small arteries and arterioles. During the early age periods, and when the adipose tissue is relatively scant, the more fibrous and closely adherent pericardial sheath with its blood vessels is apt to present a picture that may be confused with early adventitial scarring of the pulmonic trunk and with mild invasion of its wall with blood vessels.

Bearing in mind the above mentioned histological peculiarities, it may be stated that in only two of the fifty control pulmonic roots examined did obvious scarring come into consideration. In 1 case,

careful study of the supposed lesion led to the conclusion that one was dealing with an exaggerated normal variant in the distribution of the elastic, smooth muscle and connective tissue fibers. The other case, however (a child 22 months of age with hilar and meningeal tuberculosis), had to be considered as showing scarring with considerable rupture of the elastic membranes. No inflammatory cells were present and the capillaries were scant. In the inactive rheumatic cases small flame-shaped scars were seen in three instances. These were, however, very inconspicuous and no inflammatory cells were present.

Apart from the question of capillaries and scarring in the pulmonic root, there was found no significant difference in the amount of chromotropic substance as between the control and the inactive rheumatic cases. Edema was not found in either of these groups nor were eosinophilic collagen changes present.

As is to be expected, the incidence and variety of lesions in the pulmonic root is considerably greater in the active rheumatic cases. Thus, as compared to capillarization in 24 per cent of control cases and in 70 per cent of inactive rheumatic cases, this lesion was noted in 80 per cent of the active cases. In one-sixth of these capillarized cases the capillaries were quite numerous and extensive in their distribution.

Three of the active cases showed moderate sized, irregular scars — 2 with marked elastica disruption (Fig. 2) and 1 with moderate elastica disruption. In another case the elastic fibers were tremendously increased in amount and localized in small, irregular patches.

Seven of the capillarized cases showed perivascular accumulations of generally large mononuclear or lymphocytic cells, at times, polymorphonuclear leukocytes. In 6 of these there existed a diffuse inflammatory infiltration of the pulmonic root of various grades. These consisted of an irregular distribution of, at times, large mononuclear cells with large round or oval nuclei containing dust-like chromatin granules and with deeply basophilic cytoplasm. The latter was often quite abundant and possessed irregular indistinct edges. These cells generally occurred in palisades between the elastic lamellae, such as described in the aorta by Pappenheimer and VonGlabn. In 1 case, the distribution of the cells was focal, somewhat resembling the structure of an Aschoff body. Together with these mononuclear cells, or at times in their absence, there also oc-

curred an irregular distribution of neutrophilic polymorphonuclear leukocytes, lymphocytes and occasional eosinophiles. Many of the polymorphonuclear leukocytes and monocytes showed an orientation in the direction of the vessel lumen.

As mentioned before, these cells were irregularly distributed, sometimes being accumulated in greater numbers toward the inner zone of the pulmonic root media, sometimes toward the middle or outer zone, sometimes quite diffusely. In each of these 6 cases of pulmonic arteritis there were present varying degrees of eosinophilic swelling of collagen fibers and often of the elastic lamellae. These were, at times, stretched by edema fluid and accumulated inflammatory cells. In places the elastic fibers seemed to have disappeared.

In these cases and in 3 additional ones the smooth muscle cells between the elastic lamellae appeared to be swollen, with the nuclei perhaps slightly more conspicuous. As a consequence, cross-section of these fibers gave the appearance of prominent rows of cells between the elastic lamellae.

In approximately one-third of the active cases there was present some form of intimal change. In 1 case there was noted a verrucous endarteritis (Fig. 3). This consisted of the formation of typical verrucous material within a localized proliferative intimal zone. The latter was situated internally to the innermost elastic lamellae and consisted of swollen, slightly and at times deeply basophilic stellate cells separated by mucin-like groundwork. This, in all probability, represents a proliferation of the subendothelial undifferentiated mesenchyme. The structure was covered by endothelial cells except for the central portion, which was the seat of the verrucous change.

In 2 other cases there was seen a similar intimal "reduplication" but without verrucous change. In these, however, there were present swelling and eosinophilic change of the collagen with accumulations of polymorphonuclear leukocytes and lymphocytes. In the remaining cases the intimal lesion consisted of a more mature intimal reduplication, *i.e.* one in which the stellate cells have largely transformed themselves into mature fibroblasts, collagen has been laid down and some elastification has occurred. In these stages these reduplications cannot be distinguished from similar changes due to age period processes.

Summarizing these findings in the pulmonic root proper it appears that rheumatic fever produces a considerable increase in the inci-

dence and extent of capillaries, and a definite though considerably smaller increase in the incidence of scarring and genuine elastica disruption; that inactive cases show no accumulation of inflammatory cells or edema and no eosinophilic collagen changes; that in about 14 per cent of the active cases all or some of the following manifestations of acute damage are present, *viz.* accumulation of large basophilic mononuclear cells, polymorphonuclear leukocytes, formation of palisades, edema, rupture or disappearance of elastic membranes, eosinophilic swelling of collagen and elastic membranes, formation of mesenchymal intimal proliferations, formation of endarteritis verrucosa and swelling and prominence of smooth muscle cells.

PERICARDIAL SHEATH SURROUNDING PULMONIC ROOT

None of the pulmonic root sections examined from the rheumatic cases was associated with macroscopic pericarditis. In many of the peripulmonic sheaths, however, there were seen scattered lymphocytes and large mononuclear cells with some increase in the number of capillaries. This indicates a low grade irritation phenomenon. All of the active cases presented either a microscopic pericarditis of this type or an acute exudative pericarditis (8 cases) with marked accumulation of inflammatory cells and deposition of fibrin.

Of greater interest, however, were the lesions to be found in the vessels within these peripulmonic sheaths. In a previous publication Gross, Kugel and Epstein¹⁹ have classified and described a large number of lesions affecting the smaller branches of the coronary arteries in the myocardium of rheumatic cases. It will be found that a number of the vascular lesions described as occurring in the pericardial sheaths surrounding the roots of the pulmonary artery and aorta fall into the above mentioned classification. For a detailed description of these lesions the reader is referred to the above mentioned report.

The vascular changes which were found in the control series consisted of: hypertrophy of the media in 3 cases in which the terminal illness was associated with a febrile condition; intimal elastification (this occurred in 2 cases, one individual aged 22 months and one aged 74 years); intimal musculo-elastic hyperplastic changes, with the intimal smooth muscle cells somewhat irregularly arranged (this was found in 1 case, age 9 months); giant medial hypertrophy with

metallaxis (this was found in 2 cases, ages 11 and 58 years respectively). Considered as a whole, some form of vascular lesion was found in the pericardial sheath surrounding the pulmonic root in 18 per cent of these control cases.

In the inactive rheumatic series hypertrophied vessels were found in 15 per cent of the cases; intimal elastification in 1 case; typical intimal musculo-elastic hyperplastic changes in 4 cases (12 per cent) (Fig. 4); and giant hypertrophy with metallaxis in 6 cases (18 per cent). There is thus a somewhat increased incidence over the controls in the occurrence of intimal musculo-elastic hyperplastic lesions and giant hypertrophy with metallaxis. Considered as a whole, some form of vascular lesion was found in the pericardial sheath surrounding the pulmonic root in 33 per cent of the inactive cases. In the active cases the vascular lesions were far more striking. Thus, 60 per cent of the cases showed hypertrophy of the media, 18 per cent intimal musculo-elastic hyperplastic lesions, 34 per cent giant hypertrophy with metallaxis, and 18 per cent intimal fibrosis. Besides these, in 1 case there was present a necrotizing arteritis, and in 1 case (age 11 years) intimal elastification. Considered as a whole, therefore, vascular lesions were found in the pericardial sheath surrounding the pulmonic root in 66 per cent of the active cases.

Summarizing these findings in the peripulmonic sheath, it appears that inactive rheumatic cases show vascular lesions in 33 per cent of the cases, and active rheumatic cases in 66 per cent, as compared to control cases where the incidence was 18 per cent. In the inactive rheumatic group there is a moderately increased incidence of intimal musculo-elastic hyperplastic lesions and of giant hypertrophy with metallaxis; in the active rheumatic cases there is a very decided increase in the incidence of these lesions as well as of intimal fibrosis and medial hypertrophy. Exudative and necrotizing arteritis occurred in 1 case.

AORTIC ROOT PROPER

Although the outer third of the aortic media is believed to be supplied with nutrient vessels, very rare capillaries, generally confined to the medial-adventitial border, were observed in only 25 per cent of the aortic roots in the control series. In only 1 case (a child of 9 months with hilar and meningeal tuberculosis) was there present scarring of the media. This consisted of oval and flame-shaped

fibrotic lesions distributed in the middle third of the media. The elastic membranes were ruptured in the scarred areas but no inflammatory cells were present. In 1 case the sparse capillaries were surrounded by scattered lymphocytes and larger mononuclear cells.

In contrast to this the inactive rheumatic cases showed an aortic root medial capillarization in 80 per cent of the cases. In about one-third of these the capillaries were sparse, delicate and confined to the medial-adventitial border. In the remaining cases they were more numerous and penetrated deeper into the media. In these cases the vessels were generally wider in diameter and possessed a rather heavy basement membrane.

Furthermore, in 50 per cent of the inactive rheumatic cases definite scarring of the aortic root media was present. These scars were generally of four types, each more or less representing various grades of damage. A very frequent form of scarring is the flame-shaped lesion. This may be very inconspicuous, avascular and merge imperceptibly with the collagenic interdigitations of the adventitia with the media. In the definite flame-shaped scar the medial elastic lamellae are missing, capillaries are generally present and sometimes a few inflammatory cells. This scar is generally found in the outermost layers of the media, although it can occur in the middle and even inner third.

An equally frequent, decidedly more conspicuous and clear-cut lesion is the oval scar (Fig. 5). This is generally larger than the flame-shaped variety. It is oval in shape with its long axis parallel with the lumen of the aorta. It may be represented by an avascular collagenous area, free of elastic fibers. However, it generally contains capillaries, sometimes arterioles, not infrequently with swollen endothelial cells and occasionally perivascular inflammatory cells. Like the flame-shaped scar it is usually situated in the outer third of the media. This lesion may become so large as to form irregular, patchy fibrotic zones in the outer and even middle third of the media.

A very frequent and characteristic form of scarring is the moth-eaten variety (Fig. 6). This is a somewhat irregular, generally small scarred area with rather inconspicuous collagen. Its most characteristic feature is brought out by staining for elastic tissue. In such preparations it is seen that the more or less regularly ar-

ranged membranes are interrupted in patches which can be described best as moth-eaten areas. These occur generally in the outer third of the media and may be avascular and acellular. They may imperceptibly merge with what is termed the flame-shaped scar. In the latter, however, collagenous tissue is more prominent, the elastica rupture is striking and disorientation of the elastic membranes is more likely to occur.

The least frequent but most marked forms of scarring are the large irregular scars (Fig. 7). These are very obviously distorted areas of the media, often the middle third, with large irregular zones of collagenous tissue, ruptured and distorted elastic membranes and scattered inflammatory cells. They lack, however, gummatous necrosis, giant cells, conspicuous vascularization, marked perivascular lymphocytic and plasma cell infiltrations and the adventitial vascular and perivascular changes characteristic of lues. In the inactive rheumatic material 4 cases showed large irregular scars.

As mentioned before, whereas some of these forms of scarring were avascular, most of them possessed capillaries and even arterioles. The endothelium of these vessels was not infrequently swollen. There was often present a scattering of lymphocytes, large monocytes, sometimes with basophilic cytoplasm and irregular edges, and rare wandering cells of unknown type.

The aortic root media was capillarized in 85 per cent of the active rheumatic cases. In one-fourth of these the capillaries were delicate, inconspicuous, and situated at the medial-adventitial border. In the remaining cases they were of larger caliber, often with heavy basement membranes and swollen endothelial cells. Not infrequently they were associated with or replaced by arterioles. All these cases showed definite scarring. Thus, flame-shaped, oval and moth-eaten scarring occurred in approximately 40 per cent of the capillarized cases, and in 4 cases there were present large irregular scars of such dimensions as to suggest a luetic lesion.

Whereas acute exudative and destructive phenomena were absent in the inactive rheumatic cases, and inflammatory cells were sparse and confined to perivascular sites, the active rheumatic cases showed an astonishingly high incidence of these lesions. Thus, in 20 per cent of the cases, quite apart from the perivascular cellular accumulations (seen in most scarred areas), there was present a distinct inflammatory lesion of various grades of intensity often toward the inner

aspect of the media (Fig. 8) and at times irregularly situated. In a number of cases this lesion was mild and consisted of a sparse and diffuse scattering of ameboid cells with lobated nuclei, orientated very irregularly. These cells were generally of the polymorphonuclear leukocytic variety; some were larger.

In 4 cases the lesion was much more severe. In these there were present eosinophilic swelling of collagen and elastic lamellae, and development of large basophilic cells with irregular edges. These cells were found in the perivascular connective tissue of the scarred areas and also occurred conspicuously in rows between the swollen elastic lamellae (palisades).

Other cellular elements in the inflamed areas were neutrophilic polymorphonuclear leukocytes, eosinophiles, lymphocytes, large monocytes and plasma cells. At times, fragments of eosinophilic collagen were surrounded by solid-staining, irregular, elongated cells which appeared to be made up entirely of nuclear material.

Apart from these inflammatory phenomena a verrucous endarteritis was seen in 1 case. Large conspicuous rows of medial cells were seen in 10 cases, generally associated with other active inflammatory phenomena, and edema was noted in most of the acutely inflamed aortic roots. Intimal reduplications were seen in one-third of the cases. Of these, 3 showed fresh reduplications of the undifferentiated mesenchymal cell variety. Two of these possessed elastic membrane laminations (Fig. 9). In another case the intimal reduplication consisted of fibroblastic tissue permeated with capillaries, which gave it a spongy appearance (Fig. 10).

Summarizing these findings in the aortic root proper it appears that rheumatic fever produces an amazingly high incidence of capillarization, scarring and elastica disruption. The latter lesions occur in four histologically recognizable forms, *viz.* flame-shaped, oval, moth-eaten and large irregular scars. These not infrequently possess capillaries and even arterioles with swollen endothelium. In the inactive cases sparse accumulations of mononuclear cells often occur in the vicinity of the scars, but acute, exudative and destructive phenomena are absent. In 20 per cent of the active cases, however, apart from these perivascular cellular accumulations there were found acute inflammatory phenomena of various grades of intensity similar to those described as occurring in the root of the pulmonary artery. The severity of the lesions was, however, frequently con-

siderably greater than in the latter and the severe lesions occurred much more often. In 1 case the intimal fibroblastic tissue reduplication was permeated with capillaries.

PERICARDIAL SHEATH SURROUNDING AORTIC ROOT

Since most of the periaortic sheaths, particularly in the active rheumatic series, were the seat of a pericarditis, past or present, the adventitial layer was frequently quite dense because of concentration of the organized scar tissue. The adventitial layer not infrequently showed inflammatory cells of the acute, subacute and chronic variety. However, no Aschoff bodies were found in the adventitia proper. Aschoff cells and nodules were observed by Pappenheimer and VonGlahn in 5.4 per cent of 76 rheumatic cases.

The vascular lesions found in the pericardial sheath surrounding the aortic root in the control series consisted of medial hypertrophy in 2 cases, intimal elastification in 1 case and fibro-elastification in 2 cases. Considered as a whole, the pericardial sheath surrounding the aortic root presented vascular lesions in 5 cases (10 per cent) of the control series. In the inactive rheumatic series the incidence of vascular lesions in the pericardial sheath of the aorta was considerably increased. Thus, medial hypertrophy was found in 10 cases, intimal fibrosis in 2, fibro-elastification in 3, typical intimal musculo-elastic hyperplastic lesions in 7, and giant medial hypertrophy with metallaxis in 2 cases. Considered as a whole, the pericardial sheath surrounding the aortic root presented vascular lesions in 17 cases (50 per cent) of the inactive rheumatic series.

Even more striking were the vascular lesions found in the sheath around the aortic root in the active rheumatic series. Thus, medial hypertrophy was found in 33 cases (50 per cent), intimal elastification in 5, fibro-elastification in 2, typical intimal musculo-elastic hyperplastic lesions in 17 (26 per cent), giant medial hypertrophy with metallaxis in 10 (15 per cent), intimal fibrosis in 8, and granular plugged vessels in 1 case. Considered as a whole, the pericardial sheath surrounding the aortic root presented vascular lesions in 44 cases (66 per cent) of the active rheumatic series.

Summarizing these findings in the periaortic sheath, it appears that inactive rheumatic cases show vascular lesions in 50 per cent of the cases and active rheumatic cases in 66 per cent, as compared to

control cases where the incidence was 10 per cent. In the inactive group the incidence of medial hypertrophy and intimal musculo-elastic hyperplastic lesions is significantly high. In the active group medial hypertrophy, intimal musculo-elastic hyperplastic lesions, giant medial hypertrophy with metallaxis and intimal fibrosis occur in a strikingly high per cent of the cases. Granular plugged vessels were found in 1 case.

DISCUSSION

The findings presented in this report appear to be of interest for several reasons. First, they represent another contribution to our knowledge of the damage wrought by rheumatic fever in the first portion of the great vessels, sites which have been on the whole somewhat neglected by previous observers. Secondly, when present, many of the lesions described assume considerable significance when one attempts to determine the nature of various forms of valvulitis in which the inflammatory phenomena are indolent or inactive. In such instances it is not infrequently difficult to decide whether one is dealing with the end result of well known forms of endocarditis, particularly of the rheumatic variety, or whether the lesions represent a type of endocarditis *sui generis* of different etiology. In such studies it is of considerable value to search for other evidence implicating rheumatic fever. While the lesions described in this report are in themselves for the most part not specific, they nevertheless afford additional evidence on which to classify such material either as rheumatic, or in the absence of these changes, to suspect another etiology. Finally, this report serves as an additional contribution to the usefulness of the standardized method of studying hearts histologically which has already been shown to present a high incidence of pathological lesions when present.

One of the most striking findings presented in this report is the unexpectedly high incidence of capillarization, both of the pulmonic and aortic roots. That this occurs in 70 per cent and more of inactive as well as active cases, as compared to 25 per cent of the control non-rheumatic series, and that not infrequently the capillaries are larger, more irregular and of wider distribution in the rheumatic series, opens up many fields for speculation. For example, it seemed of interest to determine if the roots of these vessels in cases of luetic aortitis (particularly those involving the aortic root) presented sim-

ilar findings. An examination of 35 such cases disclosed obvious luetic lesions of the aortic media with marked scarring and disruption of the elastica, gummatous necrosis, vascularization, infiltration with lymphocytes and plasma cells and advanced endarteritic changes with perivascular mononuclear collections in the aortic mantle (chiefly adventitial). These findings, as Klotz has already pointed out, easily differentiate the luetic from the rheumatic lesions. On the other hand, in these same luetic cases the pulmonic root showed surprisingly little involvement. Capillarization and scarring were on the whole somewhat less marked than in the rheumatic series. Furthermore, when vascular lesions were present in the pulmonic sheath they were generally quite different, consisting usually of marked medial hypertrophy with exaggerated intimal fibrosis. While there was noted at times vascular change resembling the intimal musculo-elastic hyperplastic lesion found in the rheumatic series, there was little to be seen of the diversity of vascular lesions observed in the latter. It appears, therefore, that both the very florid inflammatory and destructive phenomena in the aortic root, together with the somewhat different findings in the pulmonic root and its pericardial sheath, serve to differentiate histologically lues from rheumatic fever. It has been mentioned that all of the active rheumatic cases presented in the peripulmonic sheaths either a microscopic pericarditis or an acute exudative pericarditis and that in the inactive rheumatic series many cases showed scattered lymphocytes and large mononuclear cells in this area. It seems possible that this inflammatory condition of the pericardial mantle bears a causal relation to the markedly increased capillarization of the great vessel roots. At any rate, this mechanism must be considered as a factor in addition to spread of the irritative agent by way of the vasa vasorum (Klotz) and by way of the main blood stream (Pappenheimer and VonGlahn).

While scarring is not a conspicuous feature in the pulmonic root in rheumatic fever it becomes extremely important in the aortic root where, as has been indicated by other authors, the lesions may be so extensive as to be confused with lues. In such instances the damage may undoubtedly express itself functionally by producing diminished resiliency and elasticity of the great vessel roots. The smaller lesions, however, appear to be only of histological interest.

The finding of inflammatory lesions in 20 per cent of aortic roots in the active rheumatic series was not unexpected even though the 6 per cent incidence of intimal verrucous lesions was certainly more than anticipated from gross observations. On the other hand, the 14 per cent incidence of inflammatory lesions in the pulmonic root, with 1 case showing a verrucous lesion of the intima, was surprisingly high and of considerable interest.

The intimal reduplications are important histologically only when they are quite fresh, and serve to indicate the presence of activity. The older fibrous reduplications are also found in the control non-rheumatic series.

The vascular lesions in the peripulmonic and periaortic mantles are similar to those described by Gross, Kugel and Epstein. The considerable increase in these lesions both in active as well as inactive cases is in keeping with the findings in the coronary ramifications within the myocardium proper. It is to be noted that, as in the latter, the lesions referred to as intimal musculo-elastic hyperplastic changes, giant medial hypertrophy with metallaxis and intimal fibrosis are the most significant because of their rarity in control non-rheumatic material and because of their conspicuous increase in the rheumatic series. Arteritis and granular plugged vessels were found in only 1 case each.

There remains to discuss the fate of the lesions. This question was taken up in some detail in the above mentioned report by Gross, Kugel and Epstein on the lesions of the coronary arteries and their branches in rheumatic fever, which concerned itself with the myocardial coronary ramifications. It may be said in brief that the somewhat lower incidence of the more characteristic lesions in the pulmonic and aortic roots of the inactive as compared to the active rheumatic fever series indicates that some of them may heal with little discernible residua, some may become transformed into the less characteristic alterations seen in the normal control series due to age period changes, and that the more marked lesions probably occur in patients so violently afflicted with the disease that many fail to reach the inactive stages.

SUMMARY

A variety of lesions found in rheumatic fever in the roots of the pulmonary artery and aorta together with their pericardial mantles

have been described and considered statistically. The observations were made on 66 active rheumatic hearts, 34 inactive rheumatic hearts and 50 non-rheumatic control hearts. It is shown that in both active and inactive rheumatic fever the aortic and pulmonic roots display a strikingly high incidence of destructive and inflammatory lesions consisting of scarring, elastica disruption, vascularization and other inflammatory phenomena. It is also shown that the pericardial mantles surrounding the roots of these great vessels display a high incidence of various vascular lesions. Some of these are similar to those occurring in non-rheumatic controls due to age period changes. Others, however, are similar to the vascular lesions due to rheumatic fever found in the myocardial coronary ramifications. A discussion is given of the significance of these findings.

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DESCRIPTION OF PLATES

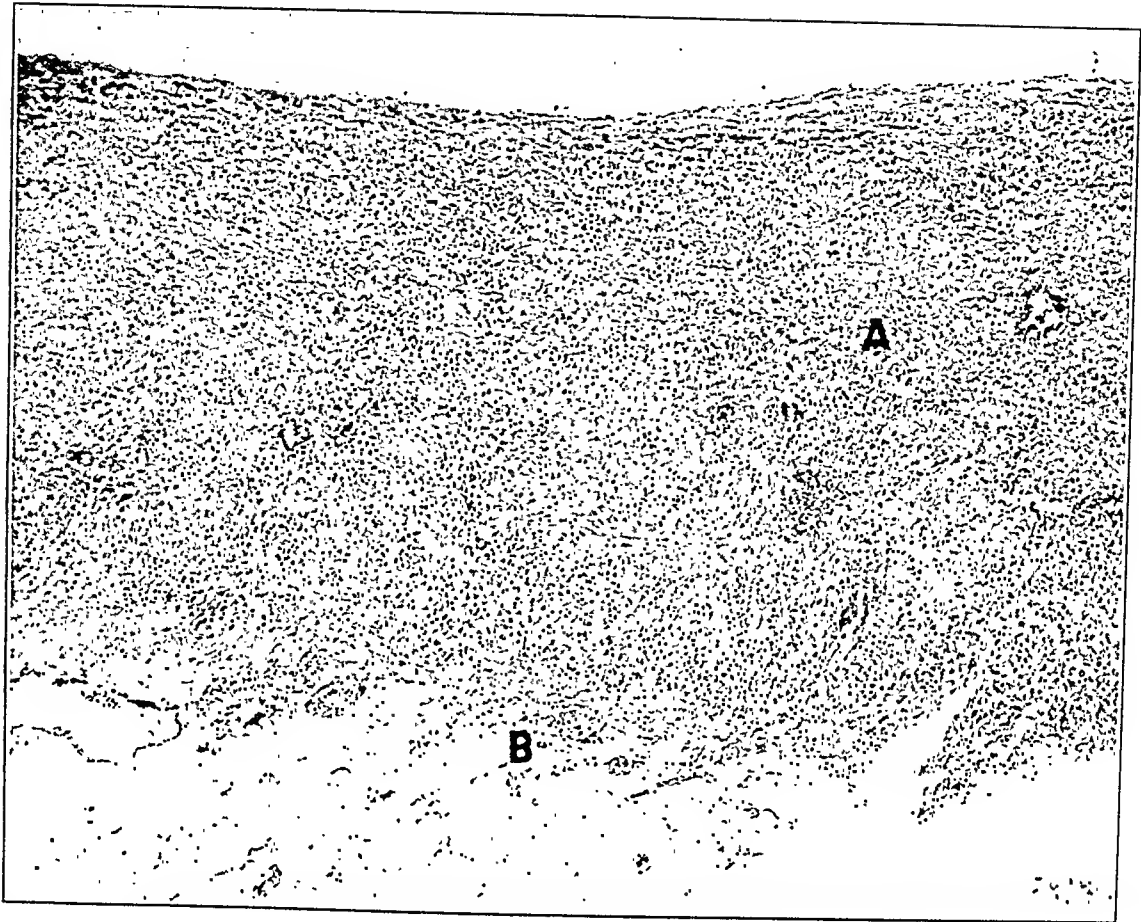
PLATE 84

FIG. 1. Pulmonary artery root from inactive case of rheumatic fever. Age 65 years. Medium power. Hematoxylin and eosin stain.

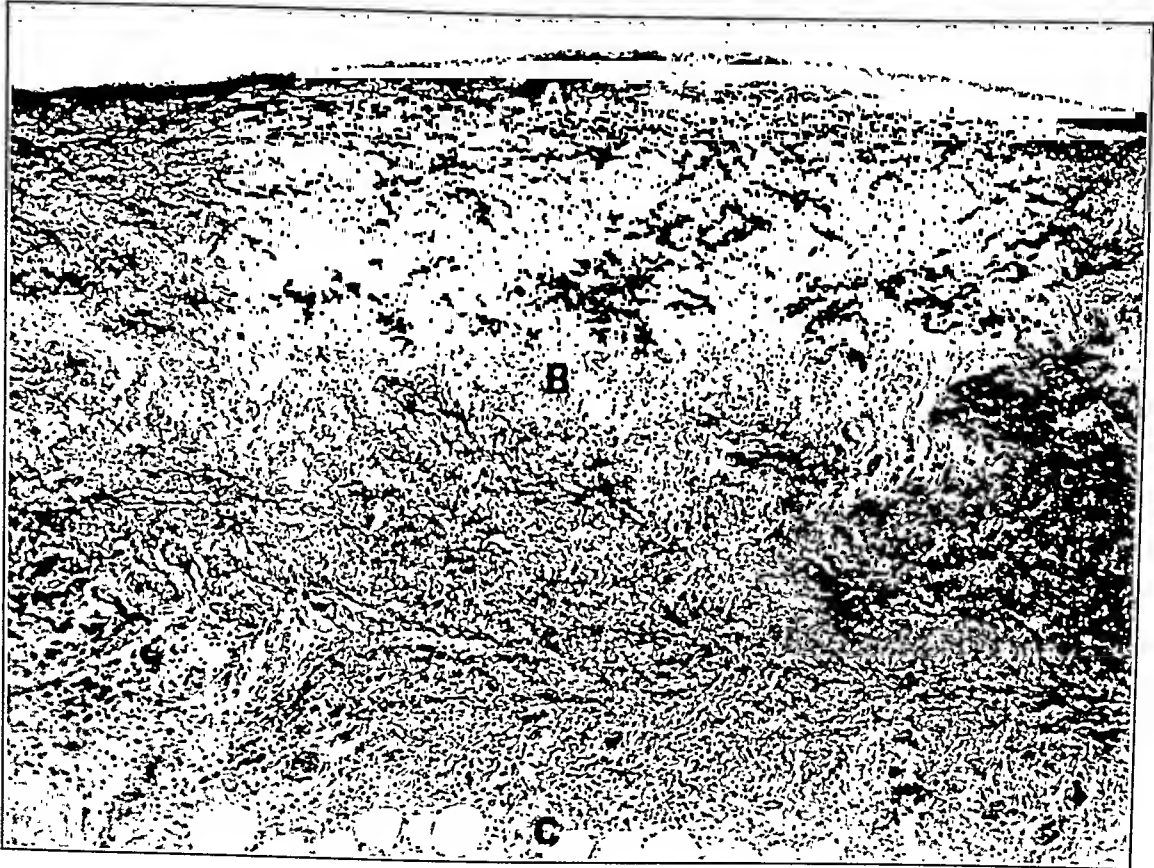
A = media showing extensive capillarization with penetration into the inner third of the vessel wall; B = adventitia.

FIG. 2. Pulmonary artery root from active case of rheumatic fever. Age 25 years. Medium power. Weigert's elastic and Van Gieson's connective tissue stain.

A = inner zone of pulmonary artery; B = marked elastica destruction, edema, capillarization and infiltration with inflammatory cells; C = adventitia showing mononuclear cell infiltration.



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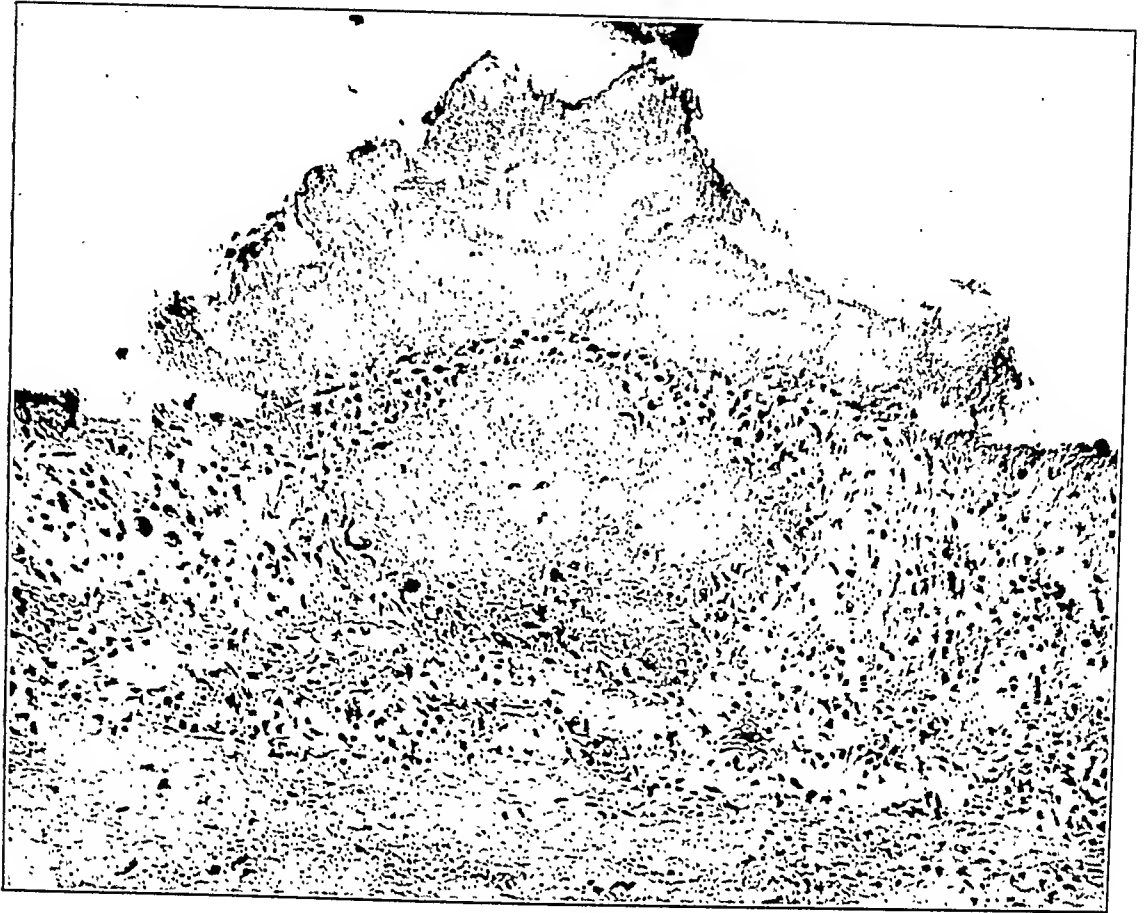
PLATE 85

FIG. 3. Pulmonary artery root from active case of rheumatic fever. Age 25 years. High power. Hematoxylin and eosin stain.

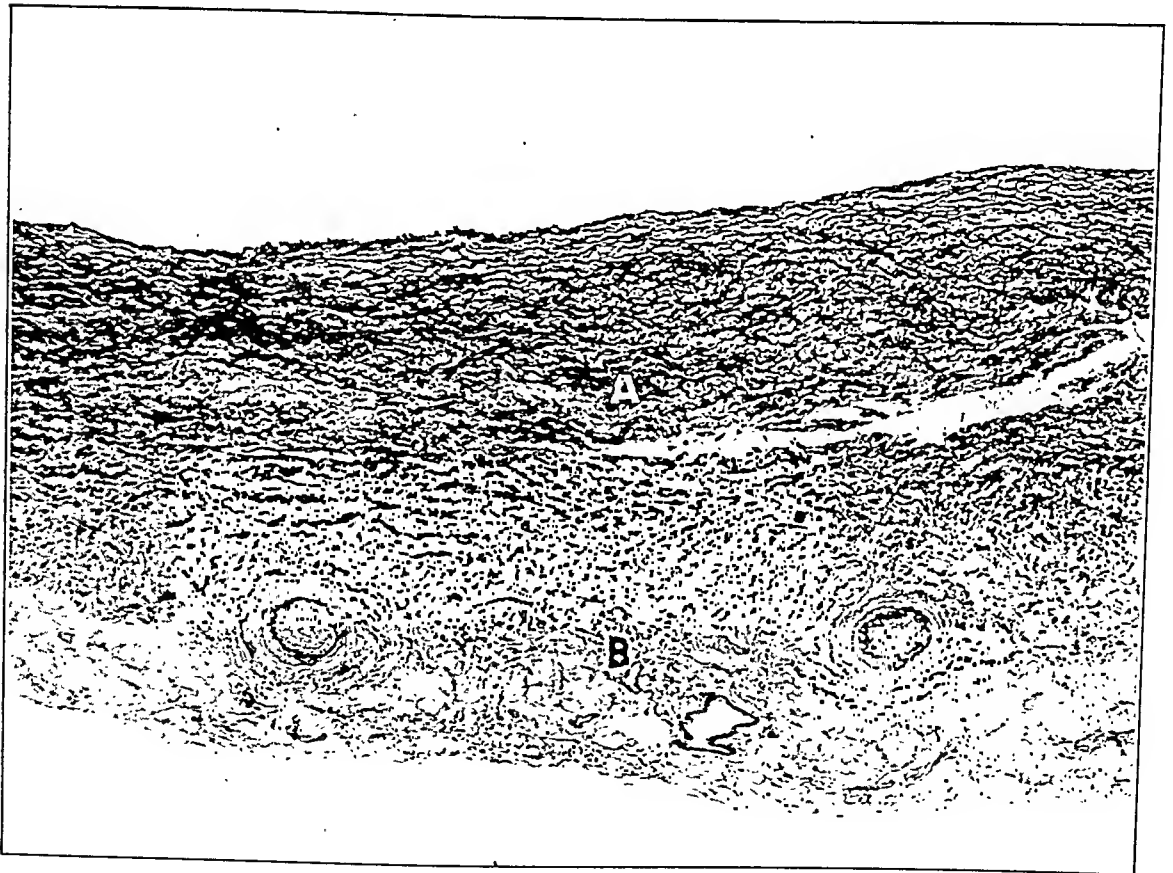
Typical verrucous formation with mononuclear cell infiltration at its base.

FIG. 4. Pulmonary artery root from inactive case of rheumatic fever. Age 37 years. Medium power. Weigert's elastic and Van Gieson's connective tissue stain.

A = media; B = adventitia showing two arteries with typical intimal musculo-elastic hyperplastic changes.



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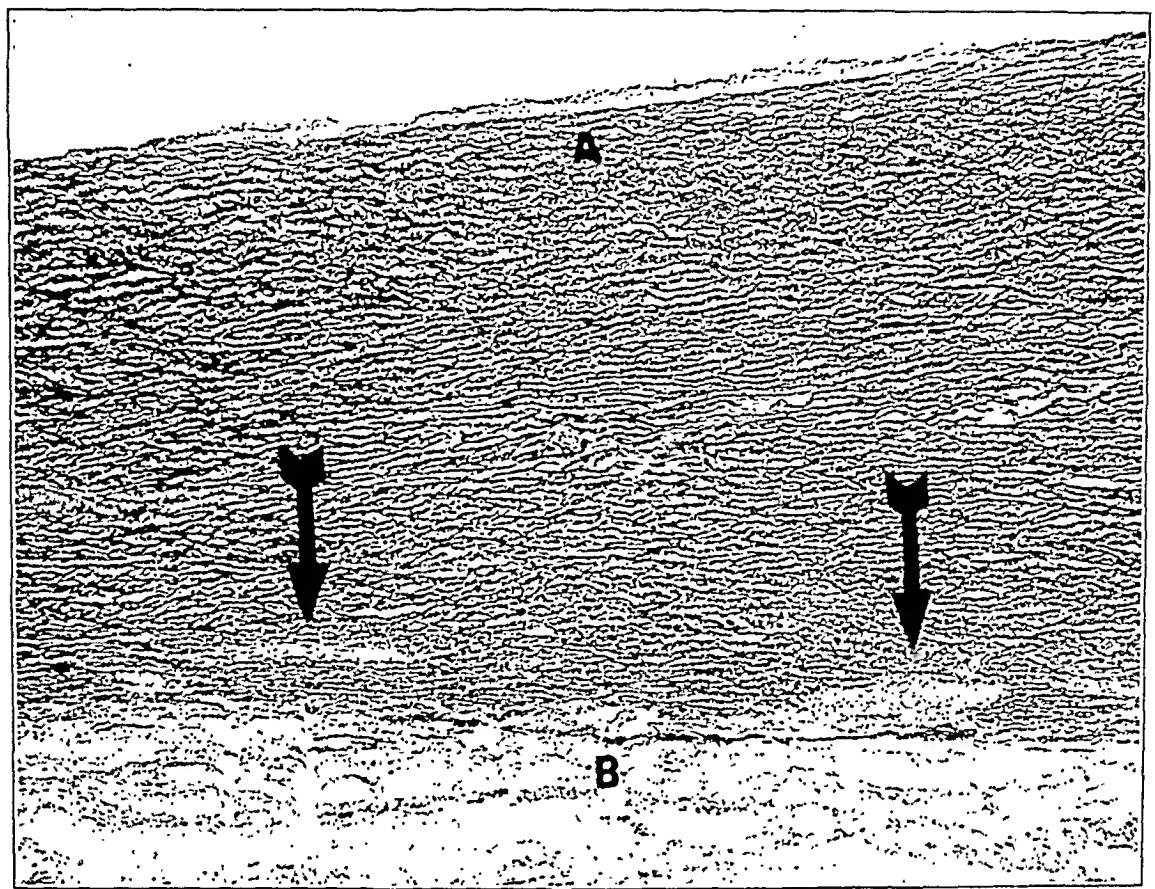
PLATE 86

FIG. 5. Aortic root from inactive case of rheumatic fever. Age 14 years. Medium power. Weigert's elastic and Van Gieson's connective tissue stain.

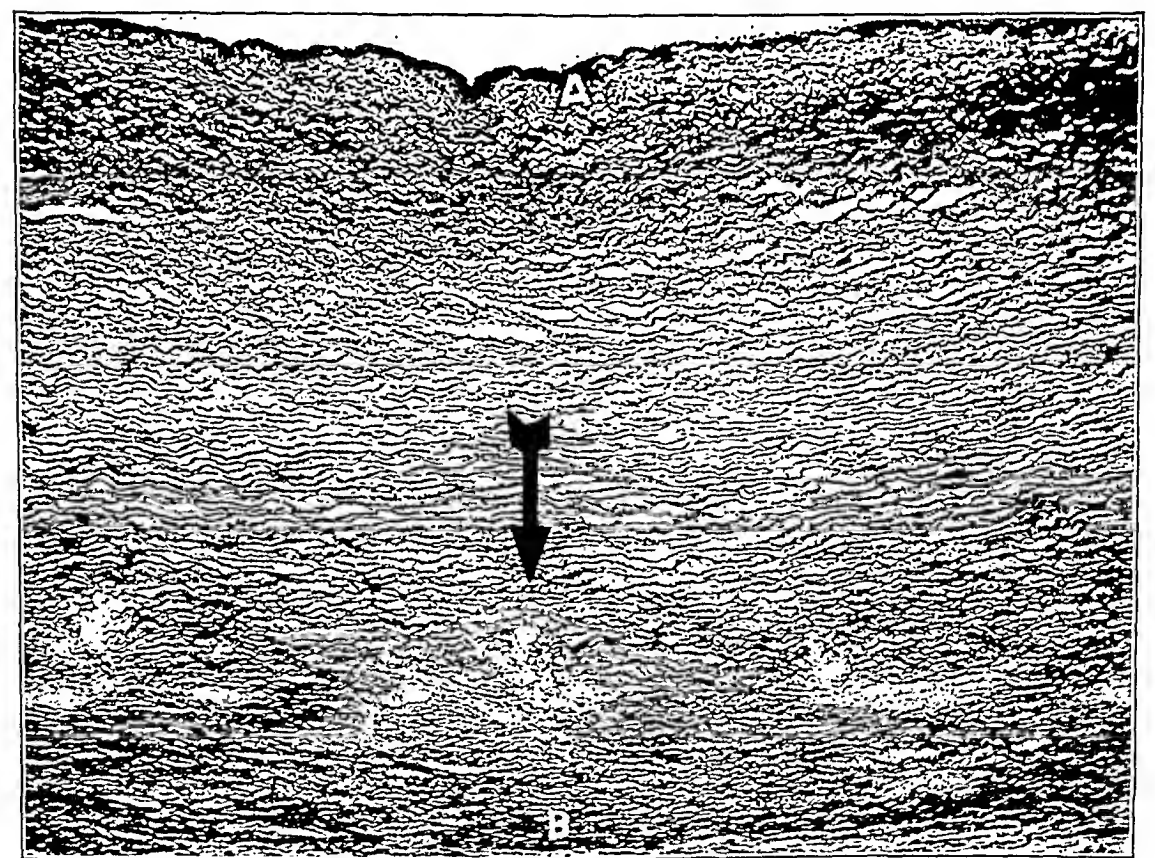
A = inner zone of media; B = adventitia. The arrows point to typical oval scars.

FIG. 6. Aortic root media from inactive case of rheumatic fever. Age 34 years. Medium power. Weigert's elastic and Van Gieson's connective tissue stain.

A = inner zone of media; B = outer zone of media. The arrow points to a typical moth-eaten scar.



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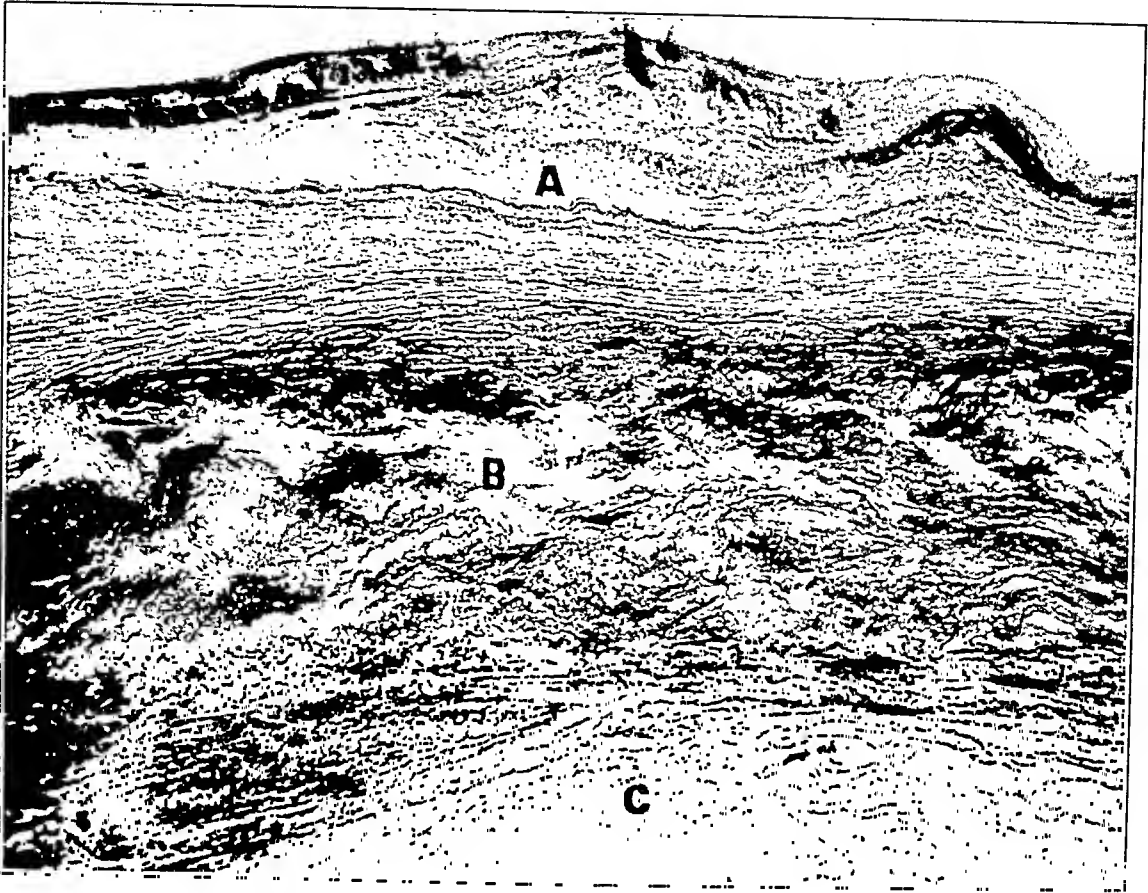
PLATE 87

FIG. 7. Aortic root from inactive case of rheumatic fever. Age 21 years. Medium power. Weigert's elastic and Van Gieson's connective tissue stain.

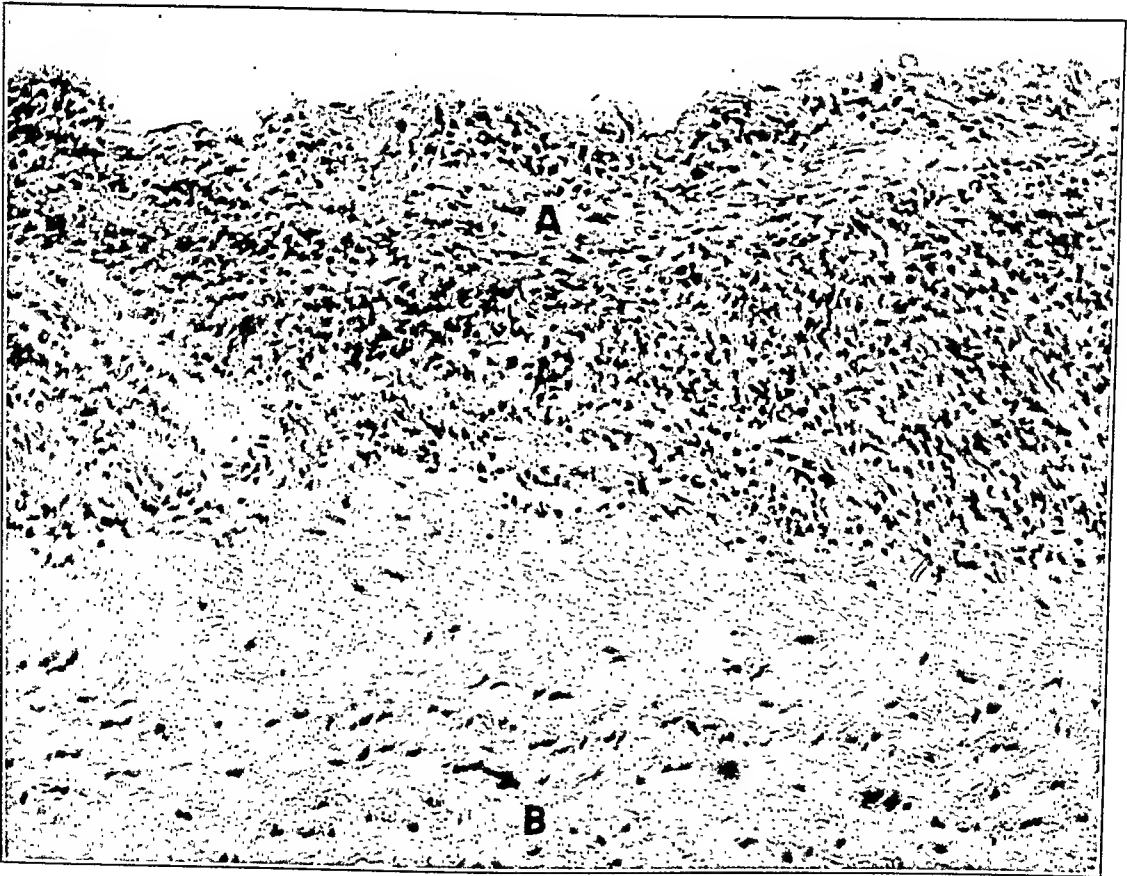
A = elastic hyperplastic and fibrotic intima; B = large irregular scarring of media with elastica disruption; C = adventitia.

FIG. 8. Intima and inner medial zone of aortic root from a case of active rheumatic fever. Age 25 years. High power. Hematoxylin and eosin stain.

A = intima and subintima showing infiltration with lymphocytes and ameboid polymorphonuclear leukocytes; B = fibrosis of inner medial layer.



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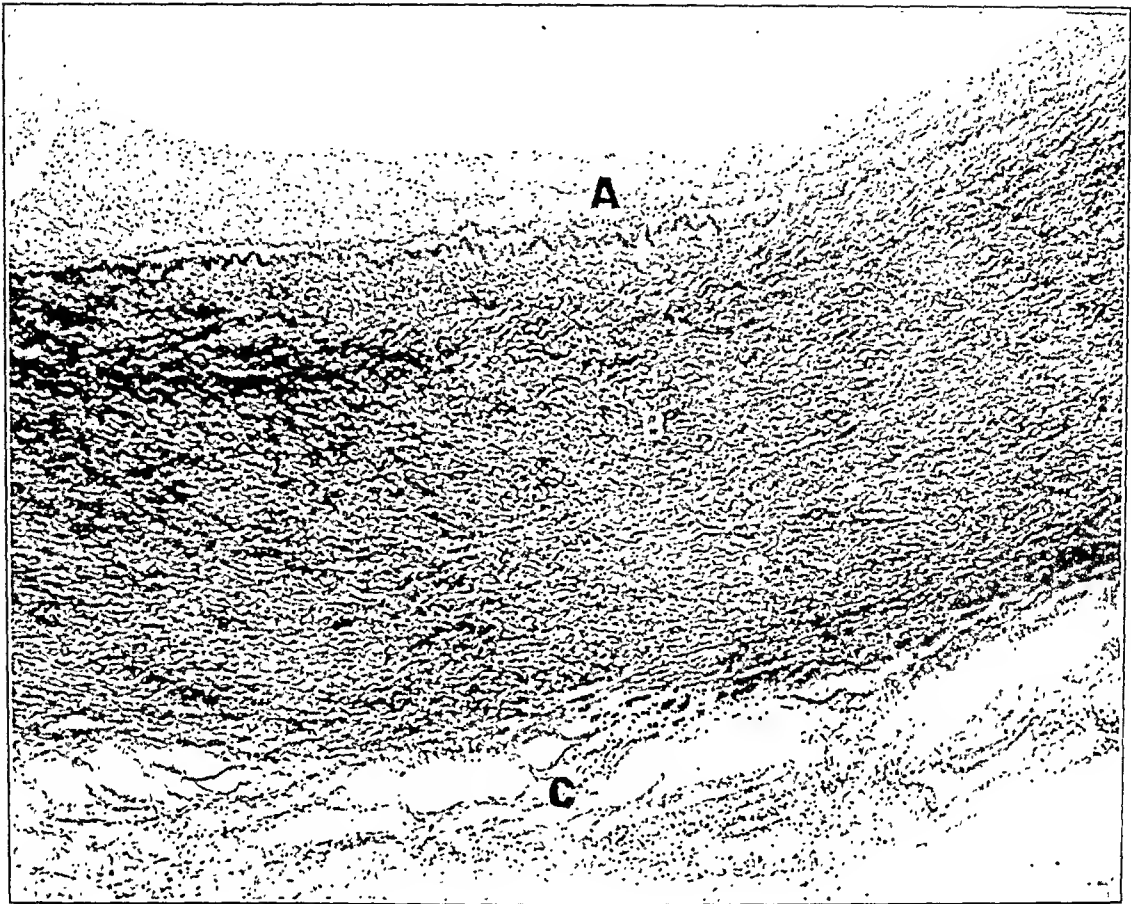
PLATE 88

FIG. 9. Aortic root from active case of rheumatic fever. Age 7 years. Medium power. Weigert's elastic and Van Gieson's connective tissue stain.

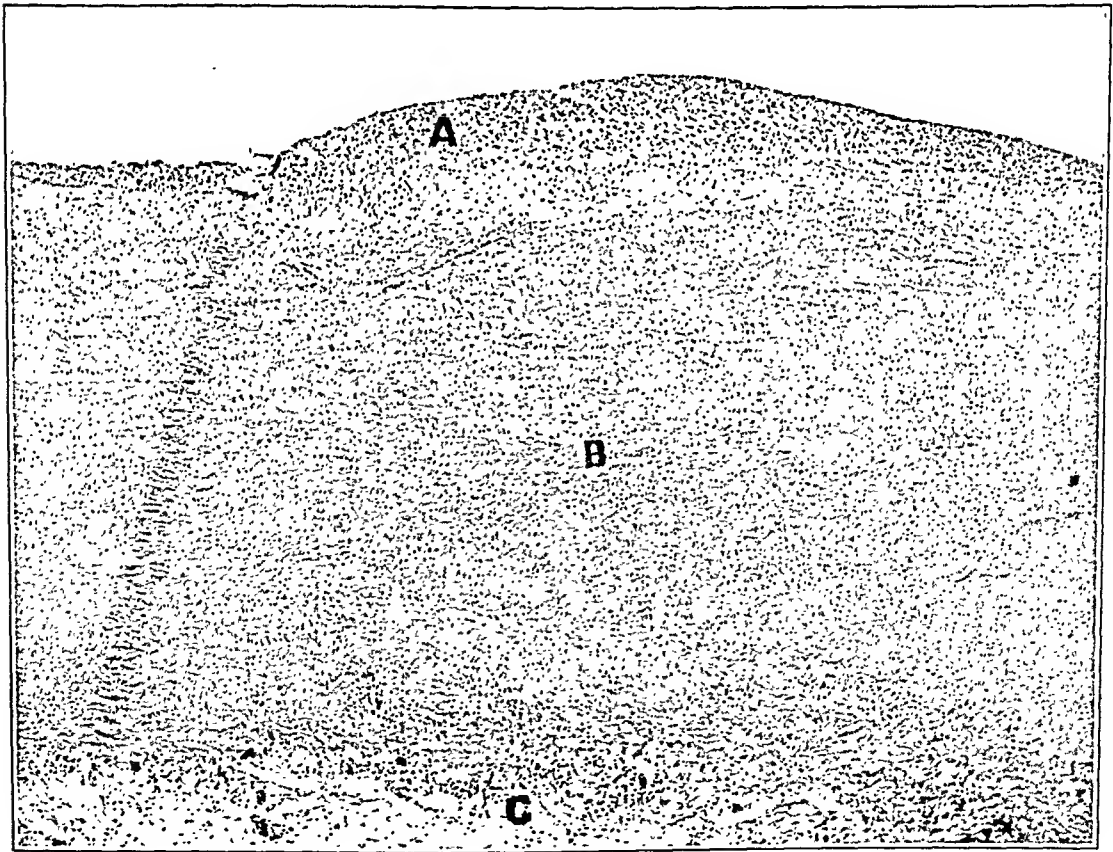
A = intimal reduplications showing elastic lamellations; B = media; C = adventitia showing mild lymphocytic infiltration.

FIG. 10. Aortic root from active case of rheumatic fever. Age 13 years. Medium power. Hematoxylin and eosin stain.

A = intimal reduplication converted into spongy mass by an interlacing network of capillaries within a fibroblastic matrix; B = media with scattered lymphocytes and polymorphonuclear leukocytes; C = adventitia with mild lymphocytic infiltration.



9



10

NUCLEAR INCLUSIONS SUGGESTIVE OF VIRUS ACTION IN
THE SALIVARY GLANDS OF THE MONKEY,
CEBUS FATUELLUS *

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Louis, Mo.)

Evidence is fast accumulating that we must recognize a special group of salivary gland viruses. All of them have been discovered by chance. They are so benign that attention was not directed to them by distinctive clinical symptoms. What attracted notice was the extraordinary hypertrophy of certain acinous or duct cells accompanied by the formation in their nuclei of inclusions resembling those caused by viruses.

The first inclusion-laden cells were reported under the heading of "protozoan-like bodies" in the parotids of two infants by Ribbert ¹ and in the submaxillary glands of guinea pigs by Jackson.² Credit is due to Goodpasture and Talbot ³ for recognizing the close resemblance between the bodies in humans and guinea pigs and for pointing out the similarity of both to the intranuclear inclusions described by Tyzzer ⁴ in varicella. Lipschütz ⁵ then rediscovered the intranuclear inclusions in herpes, admirably described and illustrated by Kopytowski,⁶ and emphasized the great importance of these bodies in "inclusion diseases" in general. But it was Kuttner and Cole ⁷ and Kuttner ⁸ who led in the demonstration that the inclusions in guinea pigs are actually caused by a virus. This naturally excited so much interest that investigators, while examining the salivary glands of other animals, have been on the lookout for nuclear inclusions, with the result that at the present time they have been reported in rats,⁹ moles,^{10, 11} mice ¹² and hamsters.¹³ Finally Kuttner and Wang ¹³ have proved that the intranuclear inclusions in hamsters, mice and wild rats are caused by viruses that are very similar to the submaxillary gland virus of guinea pigs. We await with interest proof of virus action in the formation of the inclusions in humans and moles.

* Aided by a grant from the Rockefeller Foundation for research in virus diseases.
Received for publication January 23, 1935.

OBSERVATIONS

As in the discovery of the intranuclear inclusions in the other forms mentioned, so also in *Cebus fatuellus* they were encountered almost by chance.¹⁴ A routine examination was being made of all the principal tissues of 2 *Cebus fatuellus* and 18 *Macacus rhesus* monkeys which received repeated doses of irradiated ergosterol. The inclusions were seen in the parotid and submaxillary glands of both of the *Cebus* monkeys but in none of the *Macacus*. Their appearance is illustrated in the plate.

All of the inclusion-laden cells were so much hypertrophied that they could easily be recognized without the use of an oil immersion objective. What usually caught the eye was a large, spherical, eosin-staining mass — the nuclear inclusion — with a clear halo between it and the nuclear membrane. The cytoplasm of the affected cells was distinctly basophilic, owing to the presence of cytoplasmic bodies, and consequently stood out sharply against the neighboring cells which were acidophilic, for the inclusions were limited to the secretory and intercalated ducts. None were found in the secretory acini. The altered cells were spherical in shape and bulged so far into the lumen that in some cases they seemed to occlude it.

Though easily seen, the inclusions were difficult to find by reason of their extreme rarity. A total of 33 was studied; of these 11 were seen in 102 sections of the parotid and 22 in 149 sections of the submaxillary gland. Each section was 7μ thick and about 5 mm. square. The largest number of inclusions ever seen in a single section was 4. After about a month spent in their study they could be found at the rate of 4 or 5 per hour. No inclusions were detected in the sublingual glands.

The size, and indeed all of the structural features, of the inclusions and of the cells containing them were remarkably uniform. The cells, nuclei and inclusions were roughly spherical. The diameters of 30 cells and nuclei and of 29 nuclear inclusions were measured in microns (Table I).

In the case of a binucleated cell the maximum diameter amounted to 25.2μ , but the size of the nuclei and their inclusions remained about the same. Traces of the second nucleus in the binucleated cell illustrated in Figure 6 are seen below and to the left.

All of the nuclear inclusions were strongly acidophilic when viewed

after fixation in formalin-Zenker and coloration with hematoxylin and eosin. They contained no hematoxylin-staining material. When cut through the middle they appeared to be optically homogeneous, but thin slices of their surface showed them to be made up of tiny, acidophilic adherent particles. The outlines of the inclusions were usually quite sharp. Only 1 inclusion was observed per nucleus.

The halo interposed between the inclusion and the nuclear membrane was altogether free of basophilic chromatin near the inclusion, but as one approached the membrane some particles of basophilic

TABLE I
Measurements of Cells, Nuclei and Nuclear Inclusions

Diameters	Cells	Nuclei	Inclusions
Maximum largest diameter.....	19.2	13.2	7.2
Minimum largest diameter.....	12.0	8.4	3.6
Average largest diameter.....	15.9	13.2	7.2

material could be made out — in other words, margination on the nuclear membrane was not usually complete (see Figs. 5 and 6). The width of the halo about the inclusion was approximately the same on all sides.

Nucleoli were identified in all instances where the section included a large amount of nuclear material. Invariably the nucleoli, one or two in each nucleus, left the central nuclear zone and became closely applied to the nuclear membrane. The uniformity in this migration of nucleoli, in their size (approximately 1.5 by 3 μ), degree of flattening and staining reaction was quite striking. Such a margined nucleolus is illustrated to the left side of the nuclear inclusion in Figure 3 and above it in Figures 4 and 5. The coloration indicated an even mixture of acidophilic and basophilic material, the latter almost masking the former.

Cytoplasmic inclusions were observed in 21 out of 33 nuclear inclusion-laden cells and are represented in Figures 2 to 6. They were distinctly basophilic and most of them were particulate in consistence. The largest exhibited a maximum diameter of 6 μ and a minimum of 3.6 μ ; the smallest was a sphere about 1.5 μ in diameter and the average, likewise spherical, was approximately 2.5 μ in diameter.

These cytoplasmic inclusions occurred in the distal cytoplasm between the nucleus and the lumen of the duct. Most of them were not provided with clear halos. Some, which were of much denser consistence, did have halos.

The epithelial cells next to those in which inclusions had developed showed no particular sign of injury, revealed by the simple technique mentioned, except that they were mechanically pushed aside. It may be that they were very slightly increased in size, as Scott and Pruett¹⁵ found by careful direct measurements to be the case in the submaxillaries of guinea pigs. The intercalated ducts showed no evident alterations, but the secretory ducts, which contained many more cells and in which most of the inclusions were found, did look abnormal. The cells in which nuclear inclusions had not formed did not all present the same appearance. None showed evidence of hydropic degeneration, as illustrated by Pearson¹⁶ in his Figure 12. Some were nevertheless singled out from the rest by the intense acidophilia of their cytoplasm and the pyknosis of their nuclei. Both cytoplasm and nucleus were shrunken. Such cells were observed alone and in groups, but in number they were always less than 50 per cent of the total. The lumen of one secretory duct was crowded with them. If this degeneration and desquamation of duct cells were a normal process, one would expect multiplication of the remaining cells to make good the loss, but no mitoses were seen.

Since the salivary glands of other *Cebus* monkeys, which had not received irradiated ergosterol, were not available for comparison, the best that could be done was to shift to similar tissues of *Macacus rhesus*. All of 18 given the irradiated ergosterol in equal and sometimes greater amounts exhibited the same process of degeneration and desquamation but none of them to the same degree. It was also seen in 4 untreated *Macacus* monkeys. Although these degenerating cells never showed any tendency whatever to inclusion formation, the possibility remains that their presence in such numbers is an accentuation of a normal process of elimination caused by injury to the duct epithelium and related in some way to the formation of nuclear inclusions, despite lack of topographical association between the two. We do not, however, advance this as a suggestion.

Another change in the ducts which should be reported, but not used as evidence, was accumulation of fluid about the bases (proximal poles of the cells) so that they were often quite widely separated

from the usually contiguous acinous tissue. This, also, was less marked in the *Macacus* monkeys. Calcium concretions occurred in both *Cebus* and *Macacus*, but were more numerous and larger in the former. They were probably caused or intensified by the irradiated ergosterol, as will be described in a later paper by Cowdry, Scott and Möller.¹⁷ Proliferation of fibroblasts and slight leukocytic infiltration took place near them, but the nuclear inclusions were not unusually abundant in their vicinity. Lymphocytic infiltration was often seen in the *Cebus* monkeys and less frequently in the *Macacus*. Of the 33 inclusion-containing cells in the *Cebus*, 20 were located in areas of this sort of infiltration. On the other hand, their presence in such areas was the exception rather than the rule because they were so rare and the invading lymphocytes so numerous.

The following tissues, additional to the salivary glands, were examined in each of the 2 *Cebus* monkeys to ascertain how widespread the formation of nuclear inclusions might be in the organism as a whole:

Fundus, ileum, colon, duodenum, pancreas, spleen, liver, adrenal, kidney, urinary bladder, lymph node, skeletal muscle, lung, thymus, thyroid, parathyroid, trachea, esophagus, mucous membrane of cheek, sublingual glands, testis, prostate, jejunum, pituitary, skin of back, heart muscle and bone marrow.

Since no nuclear inclusions at all like those in the submaxillaries and parotids were seen, it is evident that the reaction was restricted to these glands. Rare acidophilic droplets in the nuclei of the acinous cells of the pancreas of 1 monkey and of the pars distalis cells of the pituitary of the other were disregarded. The animals were quite mature, for their long bones had become ossified and their testicles showed active spermatogenesis. They weighed 1510 gm. and 1650 gm., respectively. Each received 6 daily doses of 5 cc. irradiated ergosterol 10,000 x (Mead Johnson and Company). All except the first of these were given with 10 gm. of calcium gluconate in tomato juice. They were killed 3 days after the last dose, at which time the blood calcium and phosphorus of 1 was 9.7 and 9.9, and of the other 9.6 and 5.

Attempts to determine if these salivary gland inclusions are caused by a virus were not undertaken because the inclusions were not discovered until weeks after the animals had been killed. Having in mind the results of transmission experiments with other salivary gland viruses, the chances of success would be good only if the

ground up glands of adults possessing inclusions were injected into animals of the same species so young that they had not acquired a natural infection and were, therefore, susceptible.

The nuclear inclusions in these *Cebus* monkeys did, however, exhibit a close resemblance to those known to be caused by viruses in the salivary glands of the guinea pig, rat, mouse and hamster. Like inclusions in the same location of moles and humans, it can only be said that their presence suggests the possible action of a virus. The nuclear inclusions in moles were more basophilic, but we found that this basophilia was at least partly due to failure of the basophilic chromatin to lose its basophilic properties or to marginate on the nuclear membrane. Instead of doing so, it accumulated in the form of a thin layer about the inclusion. The coating could easily be seen in favorable specimens. No measurements have been made, but the intensity of acidophilia of the core of the nuclear inclusion inside this basophilic layer in moles may closely approach that of the entire inclusion in *Cebus*, to which no basophilic material is attached.

In shape the inclusions are more uniformly spherical than the type inclusions in the guinea pig. The latter are often elongated in cells that are flattened. Thus, we have not found in *Cebus* any departure from the spherical shape of inclusions or cells comparable with that illustrated by Pearson.

The basophilic cytoplasmic inclusions in *Cebus* exhibited a greater range in size than in either the guinea pig or the mole. Consequently they are not so frequently disposed in orderly rows between the nucleus and the lumen. After formalin-Zenker fixation and staining with hematoxylin and eosin, the vast majority of them appeared to be made up of tiny uniform particles which look a little like *Rickettsiae*. These masses of particles were ordinarily not provided with halos. Occasionally, however, an inclusion, much more dense and not visibly particulate, was seen among the others and was limited by a halo. Very rarely all of the cytoplasmic inclusions in a single cell showed this peculiar density plus halos. Clear vacuoles may occur in the inclusions as well as in the cytoplasmic ground substance, but they were rare. When the section was overstained with hematoxylin the cytoplasm was likewise colored blue and the outlines of the inclusions could only be distinguished with difficulty. Structural details were also obscured if the sections were too thick, that is

more than 7μ . The characteristic margination and partial flattening of the nucleolus on the nuclear membrane referred to in the *Cebus* inclusion-laden cells was not encountered so repeatedly in the mole in which the nucleoli often held their spherical outlines and remained in contact with the inclusions.

DISCUSSION

Evidently *Cebus fatuellus* is to be added to the other species mentioned as exhibiting nuclear inclusions suggestive of the action of a salivary gland virus. The salivary gland viruses discovered in

TABLE II
Comparison of Salivary Gland Viruses with Pathogenic Viruses

Salivary gland viruses	Pathogenic viruses
No recognizable symptoms	Severe symptoms
Affect infants	Older individuals, infants often relatively immune
Marked cellular hypertrophy	Little or no hypertrophy
Larger inclusions	Smaller inclusions
Accompanied by basophilic cytoplasmic inclusions	Not so accompanied
Inclusion-laden cells persist for months	Are quickly removed
Active virus remains latent in tissue apparently as long as inclusions persist	Soon disappears or loses potency, for transmissions must be made promptly if they are to be successful
Virus has been transmitted only to same species	In many cases it has been transmitted to a wide range of species
Transmission only to individuals so young (generally under 1 month) that they have not become naturally infected	Adults equally satisfactory for transmission unless previously infected
Incidence in a group high, in some instances 100 per cent	Incidence comparatively rare

guinea pigs, rats, mice and hamsters, and suspected in humans, moles and *Cebus fatuellus*, are probably representatives of quite a large group, for it is likely that many others will be discovered. Even with the evidence now available it is possible to contrast these inapparent salivary gland viruses with pathogenic nuclear inclusion-producing viruses, like those of herpes and yellow fever (Table II).

The association between persistence of inclusions and presence of active virus may be stressed. Our reason for thinking that the inclusion-laden cells persist for months depends upon the age of formation, the age of disappearance and the likelihood of replacement of old inclusions by newly developed ones in the interval.

The age of formation is certainly early. Thus, Löwenstein¹⁸ found inclusions in the parotid of a 2 months old infant; Wagner¹⁹ in the sublingual of a 2 weeks old infant; Cole and Kuttner²⁰ in 3 out of 43 guinea pigs less than 1 month old; and Thompson¹² in 10 out of 70 rats, 2 months old.

Very little attention has been paid to the age of disappearance. In humans Farber and Wolbach²¹ found inclusions in 22 out of 183 infants of 17 months or less. This was a 12 per cent incidence, but 80 per cent were in individuals under 1 year of age. After 1 year they probably disappear quite rapidly, but accurate data on a large series are lacking. In "adult" guinea pigs they have been observed by many workers and in high percentages of 80 or more. It is difficult to estimate the age of adult guinea pigs ordinarily used in the laboratory but it can be taken conservatively as 1 year. Thompson failed to find inclusions in 12 rats 6 months old. Our Cebus monkeys were at least 3 years old but the age of inclusion formation is not known for them or for adult moles. The available information about adult hamsters and mice is not helpful.

With reference to replacement in humans, a statement by Farber and Wolbach is instructive: "One perplexing feature in our study of the inclusion bodies was failure to find small forms which could with confidence be interpreted as stages in formation. If the inclusions were present at all they were strikingly alike and within a narrow range of size and detail." The Rectors¹¹ in their report on moles also remarked upon the uniformity of the inclusions and the absence of young forms. The same feature was exhibited by Cebus.

It is clear from the experiments of Pearson in our laboratory that cells hypertrophied to the maximum degree and charged with nuclear and cytoplasmic inclusions are dead. The nuclear changes alone are so drastic as to appear incompatible with life. All the epithelial cells of the glands possess mitochondria in the usual number except the inclusion-laden ones from which the mitochondria have completely disappeared. Neither do they behave like living cells. When Pearson transplanted the glands of guinea pigs into the

peritoneal cavity, these cells with inclusions differed sharply from all the rest by not exhibiting autolytic changes; we think, because they were already dead.

Dead cells are as a rule promptly disposed of by the organism. It is surprising how these persist, maintaining their distinctive properties without being desquamated, falling a prey to phagocytes or being removed by autolytic enzymes. It is also interesting that we have come to rely upon such dead cells as marking the existence of active virus.

Is the virus bound to the surface of the dead cells or locked away in their cytoplasm or nucleus? If it is simply a case of adsorption on their plasma membranes the association must be a very intimate one amounting almost to chemical combination for, as pointed out by Scott and Pruett, the cells are so placed that their surfaces are being constantly washed by the passage of large volumes of water. In some species they are in acinous cells, in others in duct cells or in both. The duct cells are of the intercalated and secretory variety. It is by transfer through the walls that water is added to the secretory products. No instance is on record of the formation of inclusions by the cells of the collecting ducts, the walls of which are not regularly flushed by fluid tides in this way. Nature has indeed selected one of the worst places in the body for the attachment of virus to cells if this attachment is capable of being loosened by currents of fluid. On the hypothesis that the virus is held in the cytoplasm or in the nucleus, the immunity of the dead cells to the usual forces of disintegration would appear to favor its persistence over long periods.

SUMMARY

Intranuclear inclusions, closely resembling those caused by salivary gland viruses, were found in the parotid and submaxillary glands of 2 *Cebus fatuellus* which had received irradiated ergosterol but which showed no signs of disease. No inclusions were observed in the salivary glands of 18 *Macacus rhesus* similarly treated.

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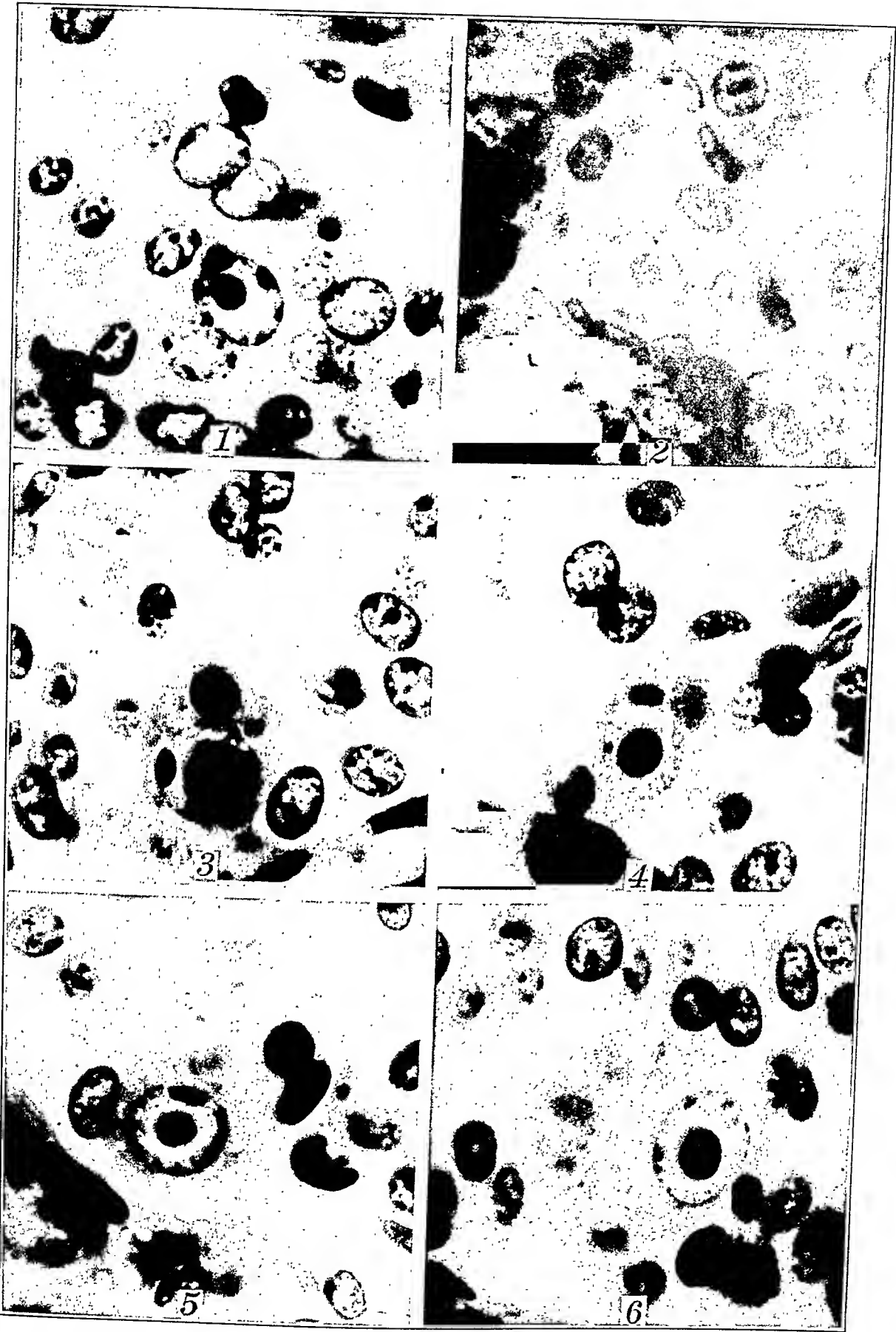
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DESCRIPTION OF PLATE

PLATE 89

FIGS. 1-6. Photomicrographs taken at a magnification of 1600 diameters of nuclear inclusion-laden cells in the salivary glands of *Cebus fatuellus*. Figs. 1 and 2, and 5 and 6 are of the parotid, and Figs. 3 and 4 of the sub-maxillary gland.



NUCLEAR INCLUSIONS IN THE KIDNEYS OF *MACACUS RHESUS* MONKEYS *

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Evidence is accumulating that the kidneys may be the site of action of inapparent viruses just as the salivary glands have been shown to be. Hypertrophied cells possessed of nuclear inclusions suggestive of virus action have been reported in the kidneys of human fetuses and young children by a number of investigators. Some of the problems raised by their discovery have been critically considered by Farber and Wolbach.¹ Intranuclear inclusions have been noted in the kidneys of London sewer rats and of *Macacus rhesus* and *Cercopithecus* sp. monkeys by Hindle and Stevenson.² They have also been found by Cowdry, Lucas and Fox³ in several other species, likewise without symptoms of disease. In this paper we shall consider the inclusions in *Macacus rhesus* particularly after the administration of large doses of irradiated ergosterol 10,000 x (Mead Johnson and Company).

OBSERVATIONS

We first encountered the inclusions in the kidneys of 12 out of 16 *Macacus rhesus* which had been treated with the irradiated ergosterol. But, instead of being very rare and of exhibiting uniform properties like those in the salivary glands of *Cebus fatuellus*,⁴ moles,⁵ rats⁶ and other forms, these renal nuclear inclusions occurred in large numbers and were highly variable. They could be arranged in series beginning with a normal cell and extending by imperceptible changes to a considerably enlarged cell with hypertrophied nucleus and conspicuous nuclear inclusion (Figs. 1 and 2). That is to say, we seemed to be dealing with a process active at the time the kidneys were fixed. There was no definite tissue reaction about the affected cells. The kidneys of 2 animals exhibited marked lymphocytic infil-

* Aided by a grant made by the Rockefeller Foundation for research in virus diseases, and an appropriation from a grant by the Rockefeller Foundation to Washington University for research in science.

Received for publication January 28, 1935.

tration, of 5 slight infiltration, and of the remaining 9 no infiltration. Some of the inclusion-laden cells showed the classical features of cloudy swelling but others did not. Desquamation of cells into the lumen was slight. One kidney exhibited marked calcium deposition but in it the inclusions were not particularly abundant. In the kidneys that showed most inclusions the nuclei of many of the tubule cells were very atypical. Some were enormously enlarged (Figs. 3 and 6), had budded extensively so that a single nucleus was replaced by 20 or more tiny ones (Fig. 5), or contained vacuoles that stained feebly with eosin (Fig. 4). A few nuclei were in process of mitotic division. The picture seemed almost neoplastic but there was no new formation of tubules. Evidence, however, of a relation between this nuclear polymorphism and inclusion formation was not forthcoming. Some of the largest inclusions occurred in nuclei that were enlarged but not atypical in structure and the most hypertrophied and atypical nuclei were sometimes devoid of inclusions (Fig. 6). The maximum weight of the animals showing inclusions was 3800 gm., the minimum, 2560 gm. and the average, 2960.9 gm. The sexes were about equally divided. They were 3 to 4 years old.

Examination of the kidneys of 10 normal controls brought to light only 1 that exhibited nuclear inclusions — an incidence of 10 per cent as compared with 75 per cent in the monkeys treated with irradiated ergosterol. To answer the question of whether or not the irradiated ergosterol was responsible for this increase we looked for nuclear inclusions in all of the kidneys of *Macacus* available in our laboratory and found them in:

16 out of 85, or 18.8 per cent of experimental poliomyelitis animals, many of which were killed before severe symptoms ensued.

2 out of 4, or 50 per cent found dead of unknown cause.

None out of 18 (acute diarrhea, 9; rabies, 1; meningitis, 1; tuberculosis, 4; pneumonia, 2; and 1 killed after injection of measles blood).

Interpretation of the results of any search of this sort for inclusions will depend upon how comprehensive it was. In this case the survey was limited to a thorough examination, with the aid of a mechanical stage, of one hematoxylin and eosin-stained section from each animal. The sections were 7 μ thick and averaged 2 sq. cm. in extent. They passed through cortex and medulla in a direction at right angles to the external (lateral) surface and midway between anterior and posterior poles. About 80 per cent of the sections in-

cluded the renal pelvis. Undoubtedly the incidence of inclusions would have been greater had more tissue been examined. But 18.8 per cent in the poliomyelitis animals and 16.8 per cent in the whole series of 107 is distinctly less than 75 per cent in the monkeys given irradiated ergosterol. The percentage for the latter was calculated on the basis of a comparable examination of a single section from each monkey. The 10 normal monkeys and the 107 employed in different experiments were of about the same size and age as those subjected to irradiated ergosterol.

Further support for the theory that the irradiated ergosterol aided in inclusion formation came from a comparison of the inclusions in animals treated and untreated with ergosterol. Among the 18 positive cases, in the 107 which did not have the ergosterol, 4 contained many inclusions with grading properties indicative of an active process and 14 showed only rare, hypertrophied inclusion-laden cells reminiscent of those in the salivary glands of *Cebus*, which point, we think, to a static condition. No more than 2 of these large cells were observed in a single section. One was binucleated and 2 were not cut in a plane favorable for making measurements. This left only 13, of which the measurements in microns given in Table I were made.

TABLE I
Measurements of Cells, Nuclei and Inclusions

Measurements	Cells	Nuclei	Inclusions
	μ	μ	μ
Maximum largest diameter	19.2	15.6	7.2
Minimum largest diameter	12.0	9.6	4.5
Average largest diameter	15.0	11.0	5.6

Evidently these measurements were not very different from those already given by us for the inclusion-laden cells in the salivary glands of *Cebus*.⁴ The inclusions were likewise wholly acidophilic. Margination of chromatin and flattening of the nucleolus on the nuclear membrane took place (Fig. 9) in the same way and apparently to the same extent. Stages in the final disintegration of the affected cells were lacking, as in the salivary glands, and cells immediately next to them showed no alterations not seen in more remote ones. Two differences, perhaps of minor importance, were

noted. The nuclei of the affected renal cells were less uniformly spherical and basophilic cytoplasmic inclusions were rarer, being detectable in 4 out of 18 cells as compared with 22 out of 33 in the salivary glands. They are best illustrated above and to the left of the nucleus in Figure 9. The point to be emphasized is that in this large series of non-treated monkeys the inclusion-containing cells in the majority of kidneys showing inclusions (14 out of 18) were all modified to about the same degree, were of rare occurrence and unaccompanied by a distinctive local reaction pointing, as we have intimated, to a process which was latent, halted or static.

A study of the distribution of inclusions was made to ascertain whether there was a significant spreading of the condition in the 12 *Macacus* given irradiated ergosterol and possessed of inclusions, as compared with the others. No inclusions were seen in the cells of the renal corpuscles or renal pelvis of any of the animals. In Table II the presence of many nuclear inclusions of variable size — our presumed active process — is indicated by a plus sign. When large hypertrophied cells were seen, the number in the section is presented in numerals 1 or 2, for there were never more. When no inclusions were seen a minus sign is inserted.

The data in the table show how much more widespread, as well as more numerous, were the inclusion-holding cells in the animals given irradiated ergosterol, but do not tell the whole story of distribution. The thin and thick segments exhibited inclusions only when situated in the cortex. The parts of these segments extending into the medulla in the Malpighian pyramids, like the collecting tubules in the pyramids, were always devoid of inclusions in this lot of animals as far as our observations went. It was unusual to find inclusions in the cortex immediately within the capsule. The inclusions were principally centered in the cortical substance at a depth from the capsule of more than 100 μ in both types of convoluted tubules, in the thick segment, initial collecting tubules and those parts of the collecting tubules in the medullary rays.

The protocols of the experiments were then studied to discover whether or not a relation existed between the dosage of irradiated ergosterol and the activity of inclusion development. For this purpose the kidneys were listed in groups depending on the number of inclusions. The data given in Table III show no evidence of such relation.

The literature abounds in references to renal lesions caused by various irradiated ergosterol or cholesterol preparations in humans,^{7, 8} dogs,^{9, 10} rats,¹¹ rabbits,^{12, 13} guinea pigs¹³ and other forms, but there is no mention of the formation of nuclear inclusions. While

TABLE II
Distribution of Inclusions

Monkeys	No.	Neck segment	Proximal convoluted tubule with medullary portion	Thin segment medullary loop	Thick segment medullary loop	Distal convoluted tubule	Initial collecting tubule	Collecting tubule	Ducts of Bellini
Treated with irradiated ergosterol	67	—	+	—	+	+	+	—	—
	89	—	+	—	+	+	+	+	—
	113	—	+	+	+	+	+	+	—
	124	—	+	+	+	+	+	+	—
	127	—	+	—	+	+	—	—	—
	134	—	+	—	—	—	—	—	—
	135	—	+	+	+	+	+	—	—
	141	—	—	—	+	+	+	+	—
	143	—	—	—	—	+	—	+	—
	146	—	+	—	+	+	+	+	—
	158	—	—	—	—	—	—	+	—
	358	—	+	—	—	+	—	—	—
Not treated	5	—	+	—	+	+	—	—	—
	10	—	I	—	—	—	—	—	—
	20	—	I	—	—	—	—	—	—
	30	—	+	—	+	+	+	—	—
	31	—	I	—	—	—	—	—	—
	40	—	—	—	—	—	2	—	—
	57	—	—	—	—	I	—	—	—
	62	—	—	—	—	I	—	—	—
	84	—	—	—	—	I	—	—	—
	110	—	—	—	—	I	—	—	—
	111	—	—	—	I	—	—	—	—
	116	—	+	—	+	+	—	—	—
	117	—	—	—	—	I	—	—	—
	119	—	I	—	—	—	—	—	—
	133	—	I	—	—	I	—	—	—
	172	—	—	—	—	—	—	—	+
	189	—	—	—	—	I	—	—	—
	234	I	—	—	—	—	—	—	—

it is possible that the inclusions might occasionally have been overlooked by individuals not actively searching for them, the uniform absence of reports of their occurrence suggests that the administration of irradiated ergosterol is not alone responsible for their development. All that can be said, therefore, is, that in our monkeys

the irradiated ergosterol may have activated or intensified a process already latent in the kidneys, as indicated by the occasional finding of inclusion-laden cells in the untreated monkeys. In the submaxil-

TABLE III

Study of Relation Between Dosage of Irradiated Ergosterol and Activity of Inclusion Development

Inclusions	No.	Total cc. ergosterol	Number of doses	Period in days	Blood calcium	Blood phosphorus
Inclusions most numerous	89	30.0	27	153	mg. per 100 cc. 17.1	mg. per 100 cc. 7.2 2 days before death
	124	5.0	1	4	12.0	..
	146	6.5	13	85	10.5 9 days before death	..
	113	11.0	20	132	9.31 2 days before death	5.6
Very numerous	67	5.0	1	3
	127	37.5	26	152	11.3	11.0 1 day before death
	135	14.0	21	151	10.7	7.7
	358	13.0	20	265	11.2	5.8
Numerous	141	10.0	2	31	11.5	..
	143	126.0	126	130	14.9 4 days before death	6.5
Rare	134	33.0	28	152	10.8	7.3
	158	33.0	28	152	18.4	8.5
No inclusions	144	5.0	1	5	11.8	..
	147	12.5	20	263	11.3	5.5
	A	30.0	3	73	12.67	8.2 1 day before death
	B	12.0	2	5	12.94	7.5 1 day before death

lary glands of guinea pigs Scott¹⁴ suppressed the development of inclusions by ligation of the duct and greatly increased it by the injection of pilocarpine, thus demonstrating the influence of experi-

mental alterations in physiological activity. The nuclear inclusions found by Pappenheimer and Maechling¹⁵ in kidney cells as the result of the administration of certain bismuth preparations are obviously different from those with which we are concerned on account of their marked basophilia and other features.

A survey was made of other tissues of these *Macacus* monkeys to determine whether the reaction of nuclear inclusion formation was restricted to the kidneys or not. In the following list of tissues the number of animals in which each was examined is given in parentheses.

Fundus (16), ileum (13), colon (13), duodenum (5), pancreas (15), spleen (15), liver (20), adrenal (15), urinary bladder (17), lymph node (11), skeletal muscle (13), lung (15), thymus (12), thyroid (13), parathyroid (8), trachea (12), esophagus (9), mucous membrane of cheek (8), ovary (8), vagina (4), uterus (7), testis (6), prostate (5), jejunum (9), pituitary (6), skin of back (5), Fallopian tube (2), heart muscle (24), cerebral cortex (4), cerebellar cortex (6), cervical spinal cord (5), cervical spinal ganglia (3), bone marrow (5), lacrimal gland (1), tonsil (2), mammary glands (2), submaxillary gland (13), parotid (11) and sublingual gland (1).

No greatly hypertrophied, inclusion-holding cells comparable to those which we have reported in the salivary glands of *Cebus* and in the kidneys of *Macacus* were encountered, but we did find nuclear inclusions of Cowdry's type B¹⁶ in the medullary cells of 11 adrenals, in hepatic cells of 4 livers, in epithelial cells of 1 lung and in the pars distalis cells of 1 pituitary. In all these tissues the inclusions were small, the cells carrying them appeared in other respects to be normal and they were of rare occurrence, although no single section of the tissues mentioned was absolutely free from them.

It will be recalled that Stewart and Rhoads¹⁷ and Covell¹⁸ have reported nuclear inclusions possibly caused by a virus in *Macacus rhesus* in the absence of clinical signs of disease. Covell discovered them in either the nasal mucous membrane, trachea, bronchioles, alveoli of lungs or bile ducts of the liver in 20 out of 60 monkeys. The highest frequency was in the pulmonary alveoli of 12 animals. He stated that no inclusions were observed in other parts of the body. The inclusions which he figured do not look much like those with which we are concerned. In the absence of transmission experiments it is idle to speculate on the relation of the inclusions we have reported in the kidneys to the others we have found elsewhere and to those of Stewart and Rhoads and of Covell.

DISCUSSION

The nuclear inclusions in the series of *Macacus rhesus* not treated with irradiated ergosterol closely resemble those that we have found in the salivary glands of *Cebus fatuellus*. In both there are: (1) great nuclear hypertrophy; (2) development of single, spherical nuclear inclusions that are acidophilic; (3) flattening of nucleolus on nuclear membrane; (4) partial or total margination of all basophilic chromatin on the nuclear membrane so that the inclusion is surrounded by a distinct and wide halo; and (5) the formation of basophilic, cytoplasmic inclusions. There is the further common feature of uniformity and rarity of the inclusion-laden cells, pointing, we believe, to a latent rather than an active process in both kidneys and salivary glands for reasons already presented.⁴ This close resemblance is compatible with the view that the nuclear inclusions in both situations are produced by a single or by two closely related viruses. In favor of the idea that one virus is acting in human infants, is the frequency of reports of similar inclusions not only in the salivary glands and kidneys but also in other parts of the body such as the lungs, liver, thyroid and so on. But Kuttner and Wang¹⁹ say that an analysis of the cases shows "that in those instances in which the submaxillary and parotid glands are reported as involved, inclusions are not found in the other tissues of the body." This line of reasoning has little force unless it is clear that all the organs concerned have been equally thoroughly examined and that negative observations are reported as regularly as positive ones. Rats may exhibit both salivary gland and renal inclusions but it is not known whether there is a coexistence of the two in single individual rats or not. In other species restriction to one locality or the other is definite and impressive:

Guinea pigs (many investigators) — salivary glands, not kidneys.

14 moles⁵ — salivary glands, not kidneys.

2 *Cebus fatuellus*⁴ — salivary glands, not kidneys.

16 *Macacus rhesus* — kidneys, not salivary glands.

The nuclear inclusions, whether in what we call the latent state characterized by uniformity and rarity in untreated *Macacus rhesus*, or in the active condition suggested by abundance and diversity after the administration of irradiated ergosterol, indicate the possibility of a virus being present in the kidneys without any attention

being called to it by any clinically recognizable symptoms of disease. From the difference in the location of the affected cells and in the appearance of the inclusions it is probable that the hypothetical virus differs from the other hypothetical virus responsible for the formation of the inclusions described by Stewart and Rhoads¹⁷ and by Covell,¹⁸ so that two inapparent viruses may exist in the *Macacus rhesus*, the monkey most frequently used for experimental purposes.

SUMMARY

Intranuclear inclusions suggestive of virus action were found in the kidneys of 12 out of 16 *Macacus rhesus* given repeated doses of irradiated ergosterol, of 1 out of 10 normal controls, and of 18 out of 107 pathological controls consisting of animals employed in the laboratory in a variety of experiments but not given irradiated ergosterol.

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DESCRIPTION OF PLATES

PLATE 90

Photomicrographs at a magnification of 1600 diameters of hematoxylin and eosin-stained sections of renal tubules of Monkey 89 treated with irradiated ergosterol, as detailed in the text.

FIGS. 1 and 2. Acidophilic inclusions of variable size surrounded by clear halos in nuclei, some of which are enlarged while others are not.

FIG. 3. A greatly hypertrophied nucleus in the lower central part of the figure which contains an inclusion.

FIG. 4. Two nuclei, hypertrophied, slightly flattened and vacuolated but without distinctive inclusions.

FIG. 5. In the upper right hand side of the tubule is a nucleus that has undergone extensive budding.

FIG. 6. Extreme degree of enlargement and polymorphism of nuclei without the formation of inclusions.

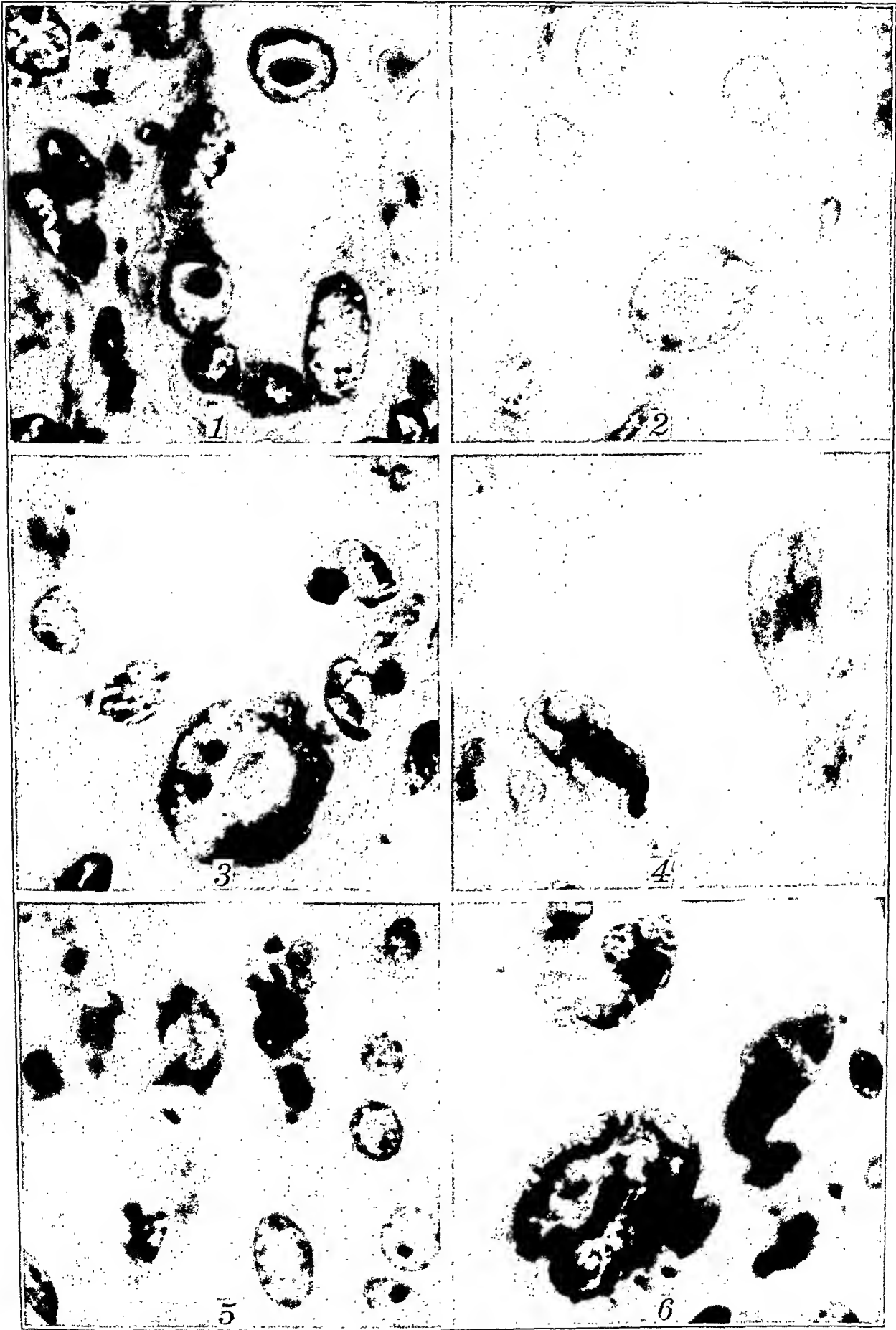


PLATE 91

Photomicrographs at a magnification of 1600 diameters of nuclear inclusion-laden cells in monkeys that were not given irradiated ergosterol.

FIG. 7. Monkey 111, experimental poliomyelitis. Single, large, spherical inclusion-containing cell.

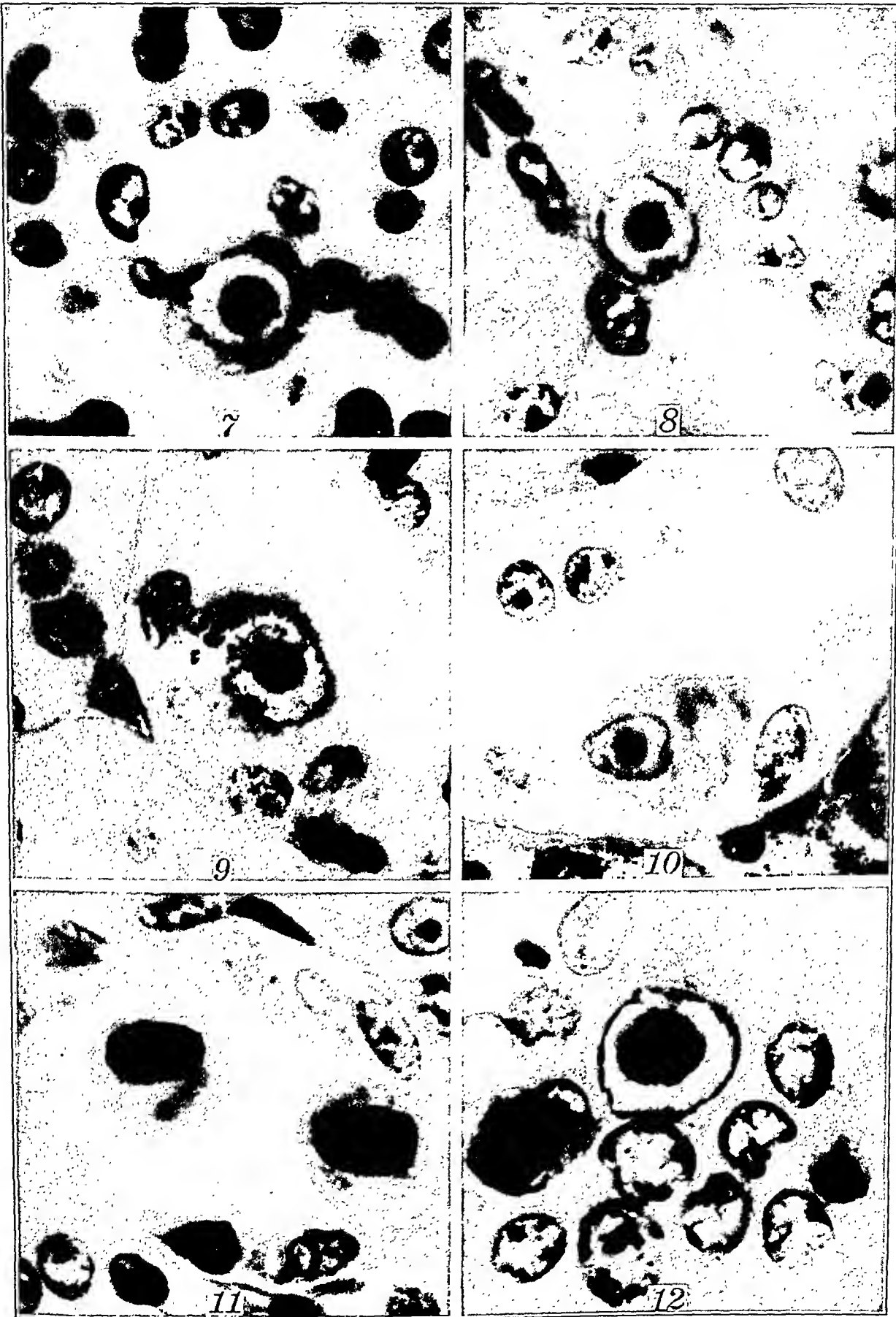
FIG. 8. Same kidney. A similar cell free in the lumen or attached to the wall at a higher or lower level in the tissue.

FIG. 9. Monkey 57, experimental poliomyelitis. Margination of the nucleolus on the nuclear membrane is evident. Basophilic cytoplasmic inclusions.

FIG. 10. Monkey 62, experimental poliomyelitis. A binucleated cell. The nucleus to the left contains a typical inclusion. The nucleus to the right is not shown because it was on a different focus. The cytoplasm was quite acidophilic.

FIG. 11. Monkey 40, cause of death unknown. Two inclusion-laden cells.

FIG. 12. Monkey 172, cause of death unknown. Epithelium of duct of Bellini cut at an acute angle with the surface. Two nuclei with inclusions.



NEUROPATHOLOGY OF EXPERIMENTAL VITAMIN DEFICIENCY *

A REPORT OF FOUR SERIES OF DOGS MAINTAINED ON DIETS DEFICIENT IN THE B VITAMINS

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INTRODUCTION

Recently a number of workers have tried to produce lesions of the nervous system in animals with diets deficient only in the vitamin B complex or in one portion of it. The divergent results of these observations ^{1, 2, 3} may be due to several causes. Diets made up of natural foods vary in composition in a manner that is at present little understood; thus, only diets made up of artificial foods can be relied upon to produce the desired vitamin deficiency. Furthermore, different species of animals vary greatly in their capacity to live on a deficient diet without manifesting symptoms. The rat, for example, if development of polyneuritis is used as a criterion, has been shown to be quite resistant to the effects of a diet deficient in vitamin B. Even animals of the same species vary in their susceptibility to deficient diets. The type of food on which the animal has been living before the beginning of the experimental regimen may account to some extent for these individual differences. Finally, the length of time that an animal lives on the deficient diet is a consideration that has not been sufficiently stressed. When an animal is completely deprived of vitamin B, it loses its appetite and may die rapidly of starvation. Under these conditions, Woollard ⁴ and, later, Kon and Drummond, ⁵ have concluded that the minor nervous system lesions which they have been able to discover can be reproduced by inanition and cachexia alone. Their animals, however, appear to have

* The expenses of this work were defrayed in part by a grant from the Proctor Fund of Harvard University for the Study of Chronic Disease.

Received for publication December 20, 1934.

been deprived of vitamin A at the same time that they were starved, and this may account for the lesions.⁶

Cowgill^{7, 8} has done extensive work in the development of artificial diets, and has produced severe nervous symptoms in dogs fed on an artificial diet almost entirely deficient in the vitamin B complex, but presumably adequate in every other respect. Goldberger and others,⁹ working with dogs, have been able to show that dried yeast contains two components, one of which is heat labile, the anti-neuritic factor, vitamin B₁ or F, and the other heat stable, which they considered the pellagra-preventing factor and which is now known as vitamin B₂ or G. Stern and Findlay¹⁰ studied two series of rats fed on diets deficient in vitamins B₁ and B₂, respectively. They found early degeneration of the myelin in the peripheral nerves and chromatolytic changes in the ganglion cells of the spinal cord when vitamin B₁ was lacking, and only vacuolization with lipochrome deposits in the ventral horn cells of the spinal cord when there was a deficiency of vitamin B₂. Because of the resistance of rats, it is surprising that these authors were able to find so much evidence of pathological changes in their animals.

Gildea, Kattwinkel and Castle,¹¹ using a diet described by Cowgill⁷ as deficient in the vitamin B complex, were able to reproduce the nervous system symptoms in dogs which Cowgill had reported as due to polyneuritis. They emphasized the fact that the symptoms suggested disturbance mainly of the central, rather than of the peripheral nervous system. In their first series of dogs maintained on the Cowgill diet, deficient in both vitamins B₁ and B₂, the affected animals were repeatedly treated with vitamin B concentrates* until they improved, and then were allowed to develop symptoms again. After a number of such therapeutic attempts the process became irreversible and large doses of the concentrate no longer brought about a recovery. Grinker,¹² working with rats, has questioned the severity of these symptoms, and the existence of a definite spastic paralysis. Cowgill⁷ published, in 1921, pictures of dogs that had developed "paralysis" while subsisting on a diet of the same type as the one employed in these experiments. In Figure 1, pictures taken from a motion picture film of the dogs studied by Gildea and his associates are presented as a final answer to Grinker's

* Yeast vitamin B (Harris Laboratories); alcoholic extract of wheat embryo (courtesy of Eli Lilly and Company). See appendix.

criticism. These illustrations, which represent the condition of the animals when therapy with vitamin B concentrates was no longer able entirely to clear up the symptoms, are necessarily inferior to the projected film, but they show spasticity of the legs, with marked disturbances of equilibrium upon walking or running. Even in the last stages of the condition, when in some animals there was complete loss of motor power of the hind legs, the knee jerks remained active, as shown in Figure 1C.

Gildea, Kattwinkel and Castle¹¹ reported that sections of the nervous systems of their dogs (Figs. 2, 3, 4 and 5) revealed evidence of myelin degeneration in the spinal cords, and in 3 out of 8 animals in the peripheral nerves. These lesions were first observed in the Weigert-Pal preparations. In order to confirm the findings, additional sections of the cords were stained with Spielmeyer's technique and corroboration of the lesions in the Weigert-stained sections was apparently obtained. Shortly thereafter Zimmerman and Burack¹ reported a study of the nervous systems of dogs that had rapidly developed symptoms on a similar Cowgill diet which was thought to be deficient in vitamins B₁ and B₂. They found demyelination of the peripheral nerves, but were unable to find lesions in the spinal cord. Moreover, they observed some evidence of degeneration in the nerves of their control animals. They pointed out that the lesions in the Spielmeyer preparations of Gildea and his associates were probably artefacts. We have found Zimmerman to be correct in his criticism of some of the Spielmeyer-stained material, but we have been unable to discover any reason to question the lesions shown in Weigert-Pal preparations.

It is noteworthy that in their first series of experiments Zimmerman and Burack made no attempt to prolong the lives of the animals, or to create a state of chronic dietary deficiency. Recently they¹³ have completed a study of the nervous system of 8 dogs which had been on a diet similar to the first one used by Gildea, Kattwinkel and Castle, except for the fact that they were given a B₁ concentrate (considered to be free of B₂) from the beginning of the experiment. In contrast to the first series of dogs studied by Zimmerman, these animals lived for as long as 300 days. In the 2 dogs of the series that died first, no changes in the spinal cord were observed. In the 6 animals that lived longer, the pathological changes consisted in a marked demyelination of the peripheral nerves,

degeneration of the medullary sheaths, and replacement by gliosis of the dorsal columns of the spinal cord, particularly the fasciculi graciles. Degeneration of the medullary sheaths of the dorsal and often of the ventral nerve roots of the cord was found, and occasionally there were slight degenerative changes in many of the other fiber tracts of the cord. In contrast to their former observations it would appear that in this last series Zimmerman and Burack have reproduced in a thorough manner the *chronic* deprivation of vitamin B which was sought in our early experiments, and so have obtained corresponding pathological changes. Their observations, however, indicate that deficiency in the vitamin B₂ component may be responsible for the development of the lesions of the nervous system. In the light of our experiments and of the results of Zimmerman and Burack, the failure of Grinker and Kandel³ to find evidence of lesions of the nervous system in rats deprived of the vitamin B complex seems probably to have been due either to the fact that these animals died in acute illness, or to the unsuitability of the species for this study.

ORIGINAL OBSERVATIONS

Since 1928 we have made four attempts to throw light on the relation of vitamin B to lesions of the nervous system, especially of the spinal cord. The particular objective was to discover whether deficiency of vitamin B had a relation to those types of "combined system disease" encountered in pernicious anemia, in pellagra, and in chronic alcoholism. Thus, the first problem was to find out whether a disturbance of the central nervous system could be produced by deficiency of vitamin B, and whether the clinical symptoms of this syndrome could be accounted for by demonstrable pathological lesions. The present report confirms the fact that this objective was attained despite the criticisms of Zimmerman and of Grinker. The second problem was to discover whether vitamin B₁ was the deficiency involved, and whether inanition played a part in the production of the symptoms and lesions. Lastly, since the clinical condition was to a certain extent reversible, an attempt was made to determine whether or not the pathological lesions could be diminished by the use of a therapeutic agent. Observations were made throughout on the relation which peripheral neuritis bore symptomatically and pathologically to the syndrome.

Series I

Series I consisted of 8 dogs that were put on a diet devised by Cowgill ⁷ (Diet I)* and supposed to be deficient in the vitamin B complex. After approximately a month of this diet all of the dogs developed anorexia, listlessness or weakness. An effort was made to maintain a chronic condition in these dogs by giving them extract of wheat embryo or yeast when marked neurological signs, often including convulsions and coma, had appeared. Except when the disturbance was too far advanced, they responded within 24 hours with an initial stage of marked general improvement. Then followed a slower second stage of recovery lasting several days, during which residual spasticity or ataxia of the hind legs was gradually relieved, but with progressive difficulty in subsequent relapses. Thus in successive relapses, after the initial relief of convulsions and coma, the hind legs especially were stiff and weak, and in some animals finally became paralyzed. Although it is difficult to be certain of sensory symptoms in animals, the behavior of these dogs, their awkwardness, and the misplacement of their limbs certainly suggested that the perception of deep sensibility was abnormal (Fig. 1). In general their reflexes were normal or hyperactive, and they appeared to have neither loss of skin sensation nor tenderness over the nerve trunks. Convulsions occurred in all but Dog 1. Tetany and opisthotonos were observed. Death finally occurred following convulsions in Dogs 2, 3, 4, 5 and 7. Dogs 1, 6 and 8 died quietly. The animals lived from 2 to 8 months.

Autopsies were done as soon after death as possible. Grossly the findings were not remarkable. The alimentary mucosa was usually injected and occasionally there were minute hemorrhages. The brain and cord were usually somewhat hyperemic. Histological study of the central nervous system showed no definite cortical or cerebellar lesions in sections stained by the Weigert method, although in most cases Nissl stains showed that the cerebral nerve cells and the Purkinje cells were degenerating. Fat was present in varying amounts in the nerve cells and perivascular spaces in the cerebral cortices of all animals. The cords showed definite myelin lesions (Weigert) in Dogs 1, 2, 3, 5 and 6 and less clear-cut lesions in Dogs 7 and 8 (Figs. 2, 3, 4 and 5). The ventral horn cells were in

* For diets, see appendix.

poor condition (Nissl), and in some instances exhibited satellitosis. Fat was present in small amounts in the nerve cells of the spinal cords of Dogs 1, 2, 5, 6 and 7. The amount of fat in all of these sections was probably not significantly greater than in normal controls. Peripheral neuritis was considered present if more than 10 per cent of the fibers in a nerve trunk contained material stainable with scharlach R or with osmic acid by the Marchi technique. By this criterion Dogs 1, 3 and 8 had peripheral neuritis.

Series II

The second series of 6 dogs was kept on the same Cowgill diet, with the addition of autoclaved yeast (Diet II). It was thought that this diet was deficient only in vitamin B₁, the antineuritic vitamin. Within a month, as with Series I, the dogs showed loss of appetite, weakness and apathy. They did not seem to respond to vitamin treatment as well as the animals of Series I. This may have been due to the fact that not so much time was spent in tube feeding and other efforts to prolong life. In general they had progressive weakness and ataxia, followed by opisthotonos and convulsions with or without tetany. No paralysis was observed. The reflexes were always present, usually hyperactive, and the dogs exhibited no flaccidity or tenderness over the nerve trunks. They lived a much shorter time (an average of 2 to 3 months less) than the animals in Series I. All of them were dead within 6 to 14 weeks after the beginning of the experiment.

At autopsy the organs appeared to be normal in gross, except for the injection of vessels in the alimentary mucosa and in the brain and cord. Dogs 2 and 6 had opacity and erosion of the cornea. Dog 6 had an apparent increase in cerebrospinal fluid. Histological study of the central nervous system showed a questionable myelin lesion (Weigert) in the cortex of Dog 1, which was corroborated by a clearly positive fat stain. A definite lesion (Weigert) occurred in the cerebellum of Dog 6, as well as a lesion of the pyramidal tract in the medulla. Nerve cells in the cortex and cerebellum were found to be undergoing degenerative changes, but to a lesser degree than in Series I. Fat was present (scharlach R stain) in small amounts in the cortices of Dogs 1, 3 and 6, and in the cerebella of Dogs 2, 3 and 6. Definite lesions (Weigert) occurred in the cord of only 1 ani-

mal, Dog 6, which lived approximately 3 months (Fig. 6). The cresyl violet-stained section from the same cord showed gliosis. Practically no fat was found. Peripheral neuritis occurred in Dogs 2 and 6. In short, only 2 of the 6 animals had significant central nervous system lesions.

Series III

Series III was designed to determine what relation inanition bore to the clinical and pathological conditions observed in Series I and II. Seven dogs (1 to 7) were placed on the original Cowgill diet, deficient in the vitamin B complex (Diet I). Three additional animals (2A, 3A and 4A) were given daily amounts of a similar diet with the addition of 4 per cent by weight of granulated unheated yeast (Diet II) equal in weight to the amount of the vitamin B-deficient food eaten the previous day by Dogs 2, 3 and 4, respectively. Dogs 4 and 4A were each given also 8 cc. of cod liver oil daily. No attempt was made to prolong life by treatment with vitamin B concentrates.

With the exception of Dog 3, all 7 of the animals that were on diets deficient in the vitamin B complex (Diet I) showed the symptoms described before — anorexia, spasticity and convulsions. In general they showed less ataxia than did the dogs of Series I and II, and their symptoms were less severe. They lived from 32 days to 4 months, and all but Dogs 1 and 5, which were found dead, were killed with chloroform. Dog 5 had had very severe symptoms and lived $3\frac{1}{2}$ months. Dog 3 showed no symptoms except mild loss of appetite. It was killed after $3\frac{1}{2}$ months, together with its control, Dog 3A. Because Dog 3 had not had much anorexia, Dog 3A had not had much inanition. Nevertheless, Dog 3A had been spastic, with exaggerated reflexes, for about a week before it was killed.

The autopsies were essentially negative in gross. Dogs 1 and 4 had corneal opacity and a purulent conjunctival discharge. Pneumonia was found in Dogs 2 and 4. In Dog 1 there was blood in the stomach and intestines. Histopathological changes in the cord were entirely absent except in Dog 5. Since this dog was found dead, and in rigor, the myelin change was probably the result of postmortem autolysis. Dogs 2, 3 and 4 showed definite peripheral neuritis, which is interesting in view of the fact that reflexes were absent in Dog 2. None of the animals showed tenderness over the nerve trunks.

Series IV

Series IV consisted of 10 dogs kept on the original Cowgill diet (Diet I) deficient presumably in the entire vitamin B complex. The experiment was designed to determine to what extent the pathological process was reversible. We hoped to demonstrate lesions in the animals that became sick and were allowed to die untreated, and either smaller lesions or none at all in the animals that were treated with tiki-tiki * during the acute phases of the illness, and with yeast (Diet II) every day during the following period. These dogs, unlike those of Series I, were not allowed to relapse repeatedly, but were treated as soon as convulsions appeared and continuously thereafter.

Four of the animals, Dogs 2, 3, 4 and 10, developed symptoms within 54 to 95 days after the beginning of the experiment. The symptoms, as before, were anorexia, spasticity, ataxia, paralysis, and finally convulsions. Therapy consisted in giving from 3 to 4 cc. of tiki-tiki in each case and in supplementing the diet from then on with 1 gm. of granulated yeast daily per pound of dog. The 4 dogs lived on this diet from 69 to 105 days, and all finally showed complete clinical recovery. They were killed with chloroform. The untreated animals, Dogs 1, 5, 7 and 8 became ill within 58 to 88 days and, with the exception of Dog 5, showed all the symptoms listed above. Dog 5 was extremely spastic after 67 days, but died without having convulsions. Dogs 7 and 8 were chloroformed. Dogs 6 and 9 showed no symptoms except a slight amount of anorexia.

The autopsies, as usual, were not remarkable upon gross inspection. Histological study showed no myelin change in any cord that we could not duplicate in our series of controls. Poliomyelopathy, consisting in disintegration of nerve cells, or in satellitosis with degenerative changes in most of the nerve cells, occurred in all of the dogs to a significantly greater degree than was found in our series of control animals. We could not demonstrate any less poliomyelopathy in the cured animals or in the symptomless dogs than in the dogs that died in acute illness. Fat stains were negative throughout. Peripheral neuritis was present only in Dog 4, and was minimal.

* See appendix.

DISCUSSION

Of the four groups of animals, Series I, given a diet deficient in the entire vitamin B complex, was the only group in which definitive myelin lesions were consistently found in the spinal cords. These dogs were the only ones in which repeated treatments with vitamin B concentrates were used to prolong life. Since in Series III and IV an exactly similar diet was given to 17 animals, but with no particular attempt to create a chronic deficiency, so that the animals died sooner, it is reasonable to conclude that a prolonged dietary deficiency is necessary in order to produce demyelination and other lesions of the central nervous system in dogs. This fact very possibly explains the failure of Grinker and Zimmerman to confirm our original observations. As mentioned above, however, Zimmerman has recently found marked lesions in dogs which were fed with great care and persistence until they finally died at the end of about 300 days. These animals were given a similar diet but with the addition of a vitamin B₁ concentrate from the beginning of the experiment. Deficiency of vitamin B₁ is thus apparently not the basis of the morphological lesions.

In Series II deficiency of vitamin B₁ was found to produce severe disturbances, with a clinical picture including convulsions and coma. As in Series I, and as had previously been observed by Cowgill,⁷ the administration of vitamin B concentrates had a rapidly beneficial effect. This result is in agreement with the belief of Findlay,¹⁴ Kinnorsley and Peters,¹⁵ and Gavrilescu and Peters^{16,17} that the syndrome produced by vitamin B₁ deficiency must, in part at least, be accounted for by a "functional" disturbance of the central nervous system, *i.e.* by one not demonstrable by our *present-day histological methods*.

In Series I and II we were not able to demonstrate lesions (Weigert) in the cortex, although in 1 animal (Dog 6, Series II) we found a definitive myelin change (Weigert) in the cerebellum. The presence of fat in the cerebral cortices, both in nerve cells and in scavenger spaces, of all the dogs of Series I and in 3 of the dogs of Series II leads us to believe that there were definite lesions present. The condition was probably not sufficiently advanced to produce myelin lesions. Cord lesions, which occurred in similar areas in a number of sections, were demonstrated by the Weigert-Pal technique in 7 of

the 8 dogs of Series I but in only 1 of the 6 dogs of Series II (Figs. 2, 3, 4, 5 and 6). It was impossible to check these lesions with any other stain. The fat-stained sections were invariably negative, and the Nissl-stained sections showed only minimal changes. Mucicarmine stains were done on both series and showed nothing abnormal. Marchi stains unfortunately were not done.

In Series III and IV, despite acute symptoms, little or no morphological evidence of myelin lesions was observed. Because of the symptoms of Dogs 3 and 3A (Series III) we conclude that either inanition may produce a small part of the clinical syndrome (spasticity), or else a diet that has an adequate vitamin content for most animals may be deficient for certain individuals. In Dogs 2, 3 and 4 of this series histological peripheral neuritis is shown to bear an entirely inconstant relation to the clinical symptoms, having been found in the 3 animals that exhibited, respectively, flaccidity, no symptoms and spasticity.

CONCLUSIONS

1. Seventeen dogs given a diet deficient in vitamin B (Cowgill) developed signs of acute disturbance of the central nervous system and died without treatment with vitamin B concentrates. Only minimal histological changes were found in the central nervous system.

2. Eight dogs given a similar diet, but whose acute neurological signs were repeatedly and temporarily relieved with vitamin B concentrates, developed gradually a residual degree of spastic ataxia and eventually motor paralysis, with reflexes present. Definitive histological lesions of the central nervous system were found in all but 1 animal.

3. Nissl stains of the cerebral and Purkinje cells and of the ventral horn cells revealed evidence of degeneration. Weigert-Pal stains of the spinal cords showed definite losses of myelin in 7 dogs. The peripheral nerves of 3 dogs showed an increase of material staining with scharlach R or with the Marchi technique.

4. The results of observations on the effect of partial starvation, of supplements of cod liver oil, and of therapy with dried yeast on morphological changes in the central nervous system were rendered inconclusive, probably because the basic deficiency was not sufficiently prolonged to produce morphological changes in the nervous system of any of the animals in such experiments.

APPENDIX

Diet I¹

20 gm. of this diet were given per kilo of body weight

	gm.
Commercial casein water-washed grade	6.3
Cane sugar	4.5
Butter fat.....	1.1
Lard	2.8
Bone ash	0.4
Salt mixture, Karr ¹⁸ *	0.2
Water	4.7

* Sodium chloride, 10 gm.; calcium lactate, 4 gm.; magnesium citrate, 4 gm.; iron citrate, 1 gm.; Lugol's solution, few drops.

Diet II

Basal diet as above, with the addition of 4 per cent by weight of autoclaved Fleischmann's yeast

Washed and purified casein from A. H. Thomas Company, and Adler Company, Philadelphia, Pa.

Dried yeast, Fleischmann Yeast Company, Boston, Mass.

Bone ash, Howe and French, Boston, Mass.

Yeast vitamin B extract, Harris Laboratories, Tuckahoe, N. Y.

Alcoholic extract of wheat embryo, Eli Lilly and Company, Indianapolis, Ind., supplied by Dr. G. H. A. Clowes.

Tiki-tiki, alcoholic extract of rice polishings, Bureau of Science, Manila, P. I.

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DESCRIPTION OF PLATES

PLATE 92

FIG. 1. Single exposures taken at short intervals from a motion picture film of Dogs 2, 5 and 6 of Series I. The pictures demonstrate the spasticity of the legs, with marked loss of equilibrium on walking or running, and the retention of the knee jerk with complete motor paralysis in the final stages of the condition.

(A) Dog 5 with wide base of hind legs and spasticity. Note that the dog is jumping clear of the ground in the second picture, and after landing on rigidly extended legs attempts to run, but falls over in a rigid attitude.

(B) Dog 5 demonstrates particularly well the spasticity of the hind legs, which are shown lifted completely off the ground in the third picture, before falling.

(C) Dog 6 in the final stage, with complete motor paralysis of the hind legs. Note, however, that the knee jerk is present, causing blur in the fourth picture.



PLATE 93

FIGS. 2, 3, 4 and 5. Photomicrographs of sections from the cords of 4 dogs of Series I which died after 4 to 6 months on a diet deficient in the vitamin B complex. Weigert-Pal stain. $\times 10$.

FIG. 2 (Dog I, Series I). Shows a symmetrical, circumscribed loss of myelin in the dorsal columns.

FIG. 3 (Dog 7, Series I). Shows a more diffuse but definite loss of myelin without symmetrical distribution except in the uncrossed pyramidal tracts along the anterior fissure.

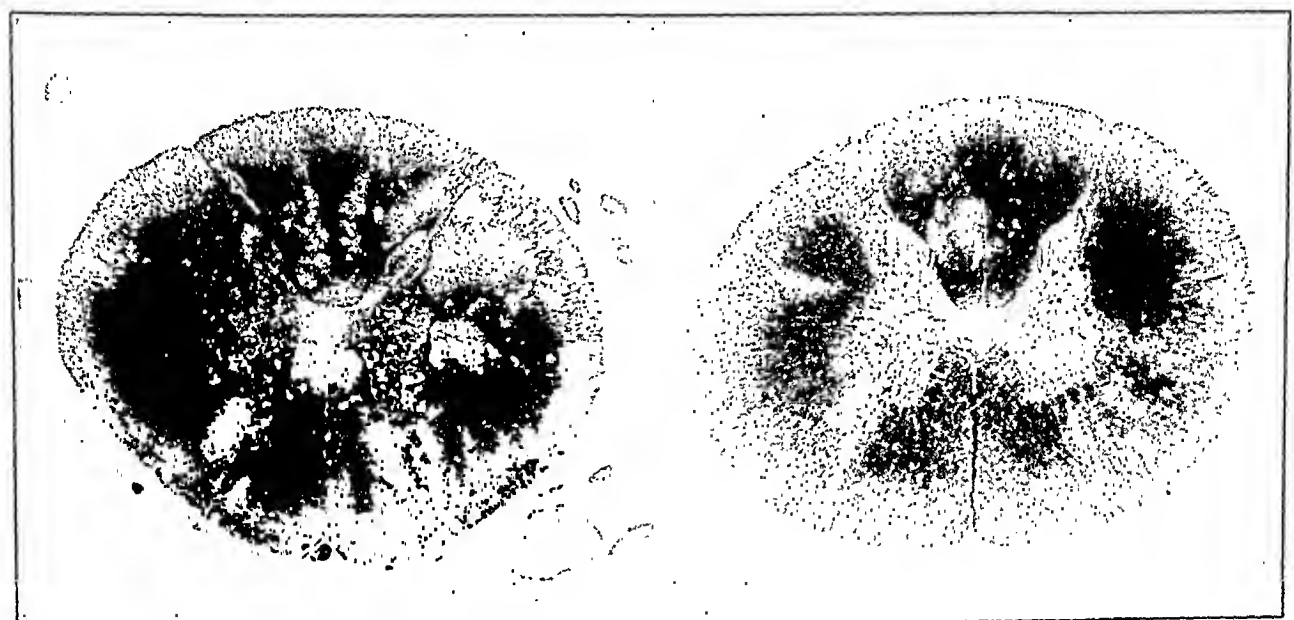
FIG. 4 (Dog 6, Series I); and FIG. 5 (Dog 2, Series I). Show definite but irregularly distributed loss of myelin.

FIG. 6. Photomicrographs of two sections from the same block of the cord of Dog 6, Series II. This dog had lived for 3 months on a diet deficient in vitamin B₁ and died in convulsions. Both sections show a loss of myelin in the same area. The same lesion was observed in fourteen other sections from the same block. Weigert-Pal stain. $\times 10$.



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MONOCYTES AS A SOURCE OF ALVEOLAR PHAGOCYTES *

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Conclusions regarding the origin of the alveolar phagocytes of the lung have been controversial since the earliest studies. The more probable sources of supply, namely, the alveolar epithelium, the macrophages of the pulmonary connective tissue, the monocytes of the circulating blood and the capillary endothelium have all been extensively investigated and championed. It has been our purpose to bring fresh evidence to the problem in an endeavor to settle the question definitely if possible. Many ingenious methods have been utilized in attacking this question, yet none has been entirely successful. Perhaps the most pertinent factor that has made the proof of the issue so difficult is the fact that no method has been unassailably successful in marking the progenitor of the alveolar phagocyte in such a fashion that its progress from the source of supply to its position as a free cell in the alveolar spaces could definitely be followed. In passing from the circulation to the alveolar space a monocyte may become marginated, migrate through the capillary wall, travel through the interalveolar stroma and pass between the alveolar lining cells to gain the air sac. In fixed tissues the cell is found at that point in its journey where it was at the moment that fixation occurred. Such a cell might, because of its location, be mistaken for a capillary endothelial cell, a resting histiocyte of the stroma, a septal cell or an alveolar epithelial cell. We have, by the method to be described subsequently, eliminated this obstacle by obtaining marked viable monocytes from one animal, injecting them into the circulation of a second animal and observing the behavior and distribution of the transferred cells in the new host.

It is not necessary again to analyze the literature that has accumulated on the subject since this has been accomplished recently by both Foot¹ and Fried.² We must, however, present briefly the prin-

* Read at the meeting of the American Association of Pathologists and Bacteriologists at Toronto, Canada, March 29, 1934.

Received for publication February 14, 1935.

cial views of various investigators in order to provide a basis for the analysis of our own experiment. They have been summarized recently by Haythorn³ in a paper on multinucleated giant cells and from that work we again quote the conclusions of leading investigators as they pertain to the issues of this problem.

The alveolar epithelium has been accepted as the origin of alveolar phagocytes by many, among whom are Sewell,⁴ Gross,⁵ and perhaps most recently, Cappel.⁶ They have reported that cells lining the alveolar spaces are able to phagocytose various suspensoid dyes injected intratracheally and have shown how these cells, having ingested various dyes, may swell, desquamate and subsequently appear as free phagocytes in the alveolar spaces. It is an indisputable point that, following the intratracheal injection of irritant dyes, one may see in the alveolar spaces many phagocytic mononuclear cells that have ingested the injected pigment. The cells may be either free in the alveolar spaces or applied to the alveolar walls. However, the evidence that these phagocytes are epithelial cells is not convincing. We have duplicated such pictures in which the rôle of the epithelium was positively excluded. In our studies we have often seen phagocytic mononuclears that had escaped from the blood stream so closely applied to the alveolar walls that they could not be distinguished from alveolar epithelium.

Gardner and Smith⁷ have studied paraffin sections of lungs vitally stained with neutral red and concluded that the phagocytes came not from endothelial or epithelial cells, but from interstitial septal cells of the alveolar walls, and that they should therefore be considered as connective tissue phagocytes. They washed free of blood the lungs which they used and concluded that they had eliminated the circulating monocytes as a source of supply. Yet it has not been proved that the cells which they saw in the interstitium had not been originally derived from the circulating mononuclears. Whence the resting histiocyte is derived is still in dispute and since Foot¹ has demonstrated that these histiocytes, together with circulating monocytes and alveolar phagocytes, have a mutual and specific affinity for certain silver dyes, we believe that one may merely represent the transitional form of another. Hence the phagocytes that appeared in their sections may have been ultimately derived from the circulating monocytes. In further support of this contention is the work of Cappel⁸ who demonstrated that marked free mononuclears in sterile

peritoneal exudates became resting histiocytes when the irritation had been permitted to subside. Fried² recently also has advanced the belief that the pulmonary macrophage or histiocyte is the progenitor of the alveolar phagocyte. He injected various irritants intratracheally and observed an abundance of cells that had phagocytosed the injected material free in the alveolar spaces. His conclusions were that the observed phagocytes were derived from a macrophage system resident in the pulmonary parenchyma itself. He, however, accepts a mesodermal origin for the alveolar lining cells and thereby automatically eliminates alveolar epithelium as a source of supply. Even if this unproved point were definitely established he does not offer proof to exclude any of the leukocytes of the circulating blood as an equally potent source of supply.

Whether or not one accepts the capillary endothelium as a possible source of the alveolar phagocyte is dependent on whether or not one believes that capillary endothelium supplies the wandering mononuclear phagocytes. Mallory⁹ was the first to propose this view and he has had many followers, among whom are Foot,^{10,11,12} Permar,^{13,14} Medlar,^{15,16} McJunkin,^{17,18} Haythorn,³ and others. Although we believe that there is strong evidence in favor of this theory we have not attempted to add any positive evidence to it in this work. We have noted many times that pigment-laden mononuclears which were injected into the circulation have appeared in the pulmonary capillaries in such positions as to render them indistinguishable from the capillary endothelium, but when simple unphagocytosed suspensions were introduced into the circulation we have never observed capillary endothelium, *in situ*, to take it up. Cappell⁶ and others have long preceded us in this observation. However, that sort of evidence does not exclude the possibility that these same endothelial cells, when released from the capillary wall, may become active phagocytes.

To our knowledge evidence in support of the belief that circulating monocytes may become alveolar phagocytes is rather sparse. Lewis¹⁹ has observed carbon-laden monocytes leaving the pulmonary capillaries to enter the alveoli of the lungs of living frogs. Foot,¹ employing his method of silver impregnation, has recently concluded that alveolar phagocytes have their origin in the circulating monocytes. We believed that if we could obtain marked viable monocytes and inject them into the circulation of a homologous animal we should

be able either to establish or eliminate them as the parent cells of alveolar phagocytes. This we have attempted to accomplish by the method about to be described. Similar methods have been employed by Seemann and Theodorowitsch,²⁰ and Borghi²¹ in their studies on the effect of the injection of homologous leukocytes in animals. Our procedure may be outlined briefly in the following steps:

1. A suspension of leukocytes was obtained by the production of a chemical peritonitis.
2. The cells were marked by a method of phagocytosis of suspensoid dyes *in vivo*. The suspension of marked cells was then removed, cleared of free pigment, washed and standardized.
3. The viability of the cells thus obtained was established by phagocytic tests *in vitro* utilizing a contrasting pigment.
4. The standardized marked viable suspension was injected into homologous animals at various points of their circulation.
5. The recipient animals were killed at various time intervals and the distribution of the marked cells was noted.

METHOD

An elaboration of the technic outlined above is as follows:

1. *Production of the Exudate:* Adult guinea pigs were used as donor animals. They yielded an exudate rich in mononuclears by the use of a single intraperitoneal injection of 5 per cent aleuronat and 3 per cent starch in plain broth.

2. *Marking and Standardizing the Cells:* At the end of 12 hours suspensoid material for phagocytosis was injected into the peritoneal cavities of the animals in which the leukocytic response had been induced for the purpose of marking the leukocytes by phagocytosis *in vivo*. The material consisted of: (a) 5 cc. of 30 per cent India ink or 5 cc. of 5 per cent lithium carmine; (b) 5 cc. of 1.5 per cent sodium citrate solution; and (c) 5 cc. of 50 per cent fresh guinea pig serum. Carbon in the form of India ink was used in the majority of cases because of its inert nature. We felt that carmine was a less acceptable substance because of its complex protein nature which we felt might be harmful to the cells that ingested it. Further, the phagocytic response of cells to carbon was greater than to carmine, probably because the carbon was present in more finely divided particles. The sodium citrate was merely used as an anticoagulant.

The fresh guinea pig serum was used because we found that its complementary action definitely enhanced phagocytosis, as can readily be seen by the data in Tables I and II. Experiments were carried out at the suggestion of Dr. G. H. Robinson,²² who had previously established this point in a series of experiments that involved phagocytic

TABLE I
*Effect of Complement on Phagocytosis in Vivo**

Amount of complement injected 50 % guinea pig serum †	Carbon-containing cells	Estimate of quantity of pigment per cell
<i>cc.</i>	<i>per cent</i>	
none	20.5	+
2	28.2	+
4	40.0	++
8	43.4	++
4	26.6	+
(inactivated)		
8	28.4	+
(inactivated)		

* Number of cells counted in each instance 200.

† Diluted with normal saline

TABLE II
Effect of Complement on Phagocytosis in Vitro

Formula	Phagocytosis observed in	Carmine-con- taining cells after 30 min.	Amount pig- ment per cell
<i>equal parts</i>	<i>min.</i>	<i>per cent</i>	
Cell suspension, 2 % carmine, normal saline ...	15	48	++
Cell suspension, 2 % carmine, 50 % complement	5	73	+++
Cell suspension, 2 % carmine, 50 % inactivated serum.....	12	56	++

tests. Our experiments were carried out utilizing both *in vitro* and *in vivo* measures. In both series we observed, as did he, that the addition of fresh complement enhanced phagocytosis with relation to three important points: (1) the increase in the number of pigment-containing cells; (2) the increase in the number of pigment particles per cell; and (3) the rapidity with which phagocytosis occurred. Fenn²³ has demonstrated that complement increases the phagocytic power of cells for inorganic substances. He explained this phenomenon on the basis of change in surface tension. We, however, believe that it is due to a stimulating action of complement on

the cells because, as is shown by the tables, when the complement is destroyed the addition of serum increases phagocytosis but slightly. Phagocytosis was permitted to progress in the peritoneal cavities of the donor animals for 12 hours. At the end of this time they were killed and the cellular exudate was removed by sterile pipettes after the abdominal cavities had been opened. Having obtained a suspension of marked cells we were still beset by the problem of clearing it of free pigment particles, inasmuch as it was essential not to inject any free pigment into the recipient animals. Curiously enough, it was found that simple passage through a paper filter yielded a filtrate which contained no free dye particles. The filtration was carried out in a dark incubator at 37° C. Fenn²³ has shown that this is the optimum temperature for maintaining the viability of cells and Earle²⁴ has demonstrated the destructive action of light on such suspensions. Why dye particles, infinitely smaller than cells, were retained on the filter, whereas the cells passed into the filtrate, can probably be explained by the theory of electrical charge in filtration, as advanced by Mudd²⁵ and others. As applied to our observations, the dye particles possessed a charge opposite to that of the filter and therefore adhered to it, whereas the cells possessed a like charge and passed through the pores without difficulty.

In order to make injection of the suspension practical it had to be concentrated to small bulk. Centrifugation did not damage the cells appreciably when carried out at low speed with the balancing cups containing water warmed to 40° C. The supernatant fluid was poured off and the cells were suspended in 50 per cent guinea pig serum. Standardization consisted of counting the cells of such a concentrate and diluting it so that each cubic millimeter contained approximately 50,000 cells. By making differential smears it was found that more than 80 per cent of the pigment-bearing cells were phagocytic mononuclears.

3. *Method of Establishing Cell Viability:* This was accomplished by observing whether the pigment-bearing cells would phagocytose a contrasting pigment or not. The tests were carried out *in vitro* in an attempt to establish a state as closely simulating body conditions as possible. Therefore, hanging drop preparations suspended in guinea pig serum were observed on a warm stage. Agitation of such a preparation, it was found, increased the rapidity of phagocytosis. We felt, therefore, that the factor of speed of circulation could be

neglected. Hanging drop preparations were made using, for example, a loopful of carbon-containing cells, a loopful of 2 per cent carmine, the contrasting pigment suspension, and two loopfuls of guinea pig serum. Observations were directed toward four major points: (1) the percentage of all cells which phagocytosed carmine particles; (2) the ability of the carbon-containing cells to take up the contrasting pigment; (3) the length of time which elapsed before general phagocytosis began; and (4) the degree of phagocytosis at the end of 30 minutes. Analysis of a series of such preparations yielded the following averages. Phagocytosis was observed within 5 minutes and 73 per cent of all cells were seen to contain carmine particles at the end of 30 minutes. The majority of cells ingested so much carmine that their structure was obscured. Those mononuclear cells that were carbon-containing were equally as active as those that were free of carbon in respect to their avidity for carmine. This established the fact that over 50 per cent of the marked cells to be injected were viable. It is reasonable to assume that this percentage would be considerably higher under absolute body conditions.

4. *Method of Injection:* To rule out anthracosis several series of recipient animals used consisted of young guinea pigs raised outside of the city. Control sections of the lungs of similarly reared animals of the same age showed only widely separated particles of carbon. In one series the cell suspension was introduced into the jugular vein, in another directly into the heart, and in a third series into the portal vein. A control group received a pure suspension of India ink in normal saline solution. In regard to this group we found, as have many others, that suspensoid dyes such as India ink when injected intravenously possess no powers of diffusion. They are stored within the cells of the so-called reticulo-endothelial system and are never found in the lining cells of the alveoli or in the alveolar spaces in any appreciable amount.

5. *Preparation of the Histological Specimens:* The animals were killed with chloroform at intervals varying from 3 hours to 7 days. Sections were fixed in formalin and Zenker's fluid. The stains employed were eosin-methylene blue, Wright's, and simple hematoxylin. Unstained sections were also prepared. The simple hematoxylin method gave a very delicate stain in which carbon-containing cells contrasted sharply with cells free of the pigment.

RESULTS

The microscopic picture of all animals was almost identical regardless of the time permitted to elapse before they were killed or the site at which the suspension was injected.

The dye-containing cells were found almost entirely in the lung tissue. Some were seen within the pulmonary capillaries, some appeared in the lung stroma, but the vast majority were found to be either free in the alveolar spaces or applied to the alveolar walls, apparently lining the alveolar walls and thereby presenting the morphological picture of alveolar epithelium. In several instances pigment-bearing mononuclears were seen escaping from pulmonary capillaries, the bulk of their cytoplasm being adherent to the alveolar walls in the position of epithelium and the remainder of the cell still within the capillary. Had we not known their origin we would have interpreted many of these cells as swollen epithelial cells which had ingested dye granules. Still others, situated in the connective tissue, would probably have been considered to be histiocytes of the stroma.

The liver, spleen and bone marrow contained occasional dye particles. We are aware of the fact that some pigment-bearing cells were no longer viable when introduced into the circulation. Naturally they were disrupted with consequent liberation of their pigment. This free pigment was then taken up by the phagocytic system of the liver, spleen and bone marrow.

DISCUSSION

We found that viable phagocytic mononuclears marked by the ingestion of pigment, when injected into the circulation of a guinea pig, were concentrated in the lungs. There they were identified as alveolar phagocytes, although those in the intermediary locations between capillary and alveolar space may well have been identified as capillary endothelium, macrophages or septal cells, had we not known them to be carbon-laden mononuclears which we injected into the circulation. The suspected progenitor, namely the phagocytic mononuclear, was found to appear as the alveolar phagocyte.

It is impossible to state, at present, the reason for the concentration of these cells in the lung. We can say, however, that it was not

due to simple capillary filtration for those cells injected into the portal vein were not filtered out in the liver but appeared in the lungs. Furthermore, marked cells were identified in differential smears from the circulating blood as much as 2 hours after their injection.

It occurred to us that perhaps the cells which we injected were destroyed in the blood stream of the recipient animals; that the liberated pigment was then phagocytosed by certain cells residing in the pulmonary parenchyma of the recipient. But this is impossible because suspensoids have no power of diffusion. When a solution of India ink was injected we were unable to discover any of it in the alveolar spaces or in cells lining their walls.

We have not attempted to establish monocytes as the sole origin of alveolar phagocytes under all conditions. Cunningham, Sabin and Doan,²⁶ and others grant monocytes a histiocytic origin. If this relationship is definitely established the problem of the alveolar phagocytes will be solved indisputably.

SUMMARY AND CONCLUSIONS

1. A technic for preparing a marked cell suspension in one animal and transferring it to other animals is described.
2. The complemental power of serum enhances the phagocytic properties of homologous leukocytes *in vivo* and *in vitro* by increasing the number of pigment-containing cells, the number of particles per cell and the rapidity of the process.
3. Viable phagocytic mononuclears marked by the ingestion of pigment are concentrated in the lungs regardless of the site of injection of the cell suspension.
4. Alveolar phagocytes are derived, largely if not entirely, from monocytes of the circulating blood.

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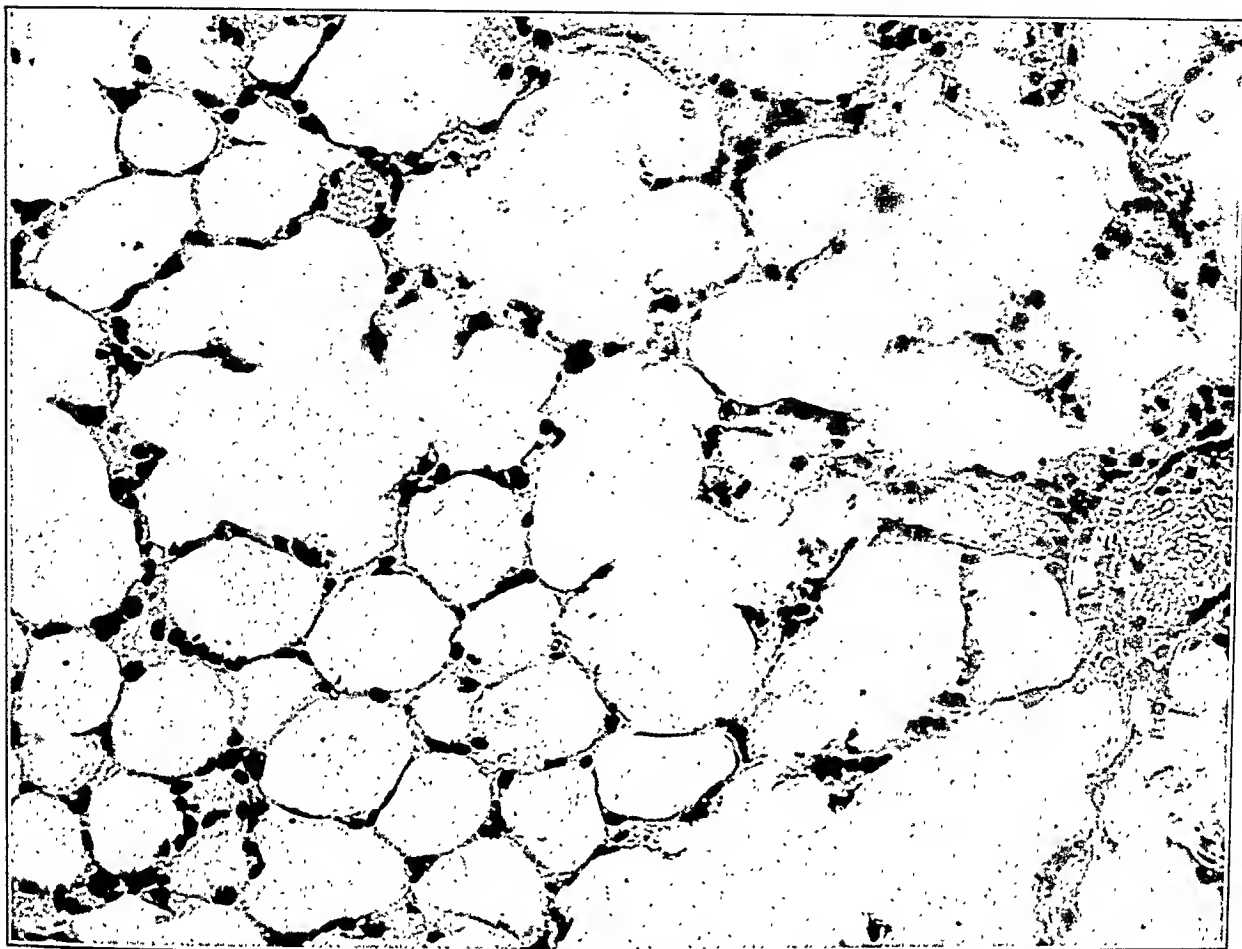
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DESCRIPTION OF PLATES

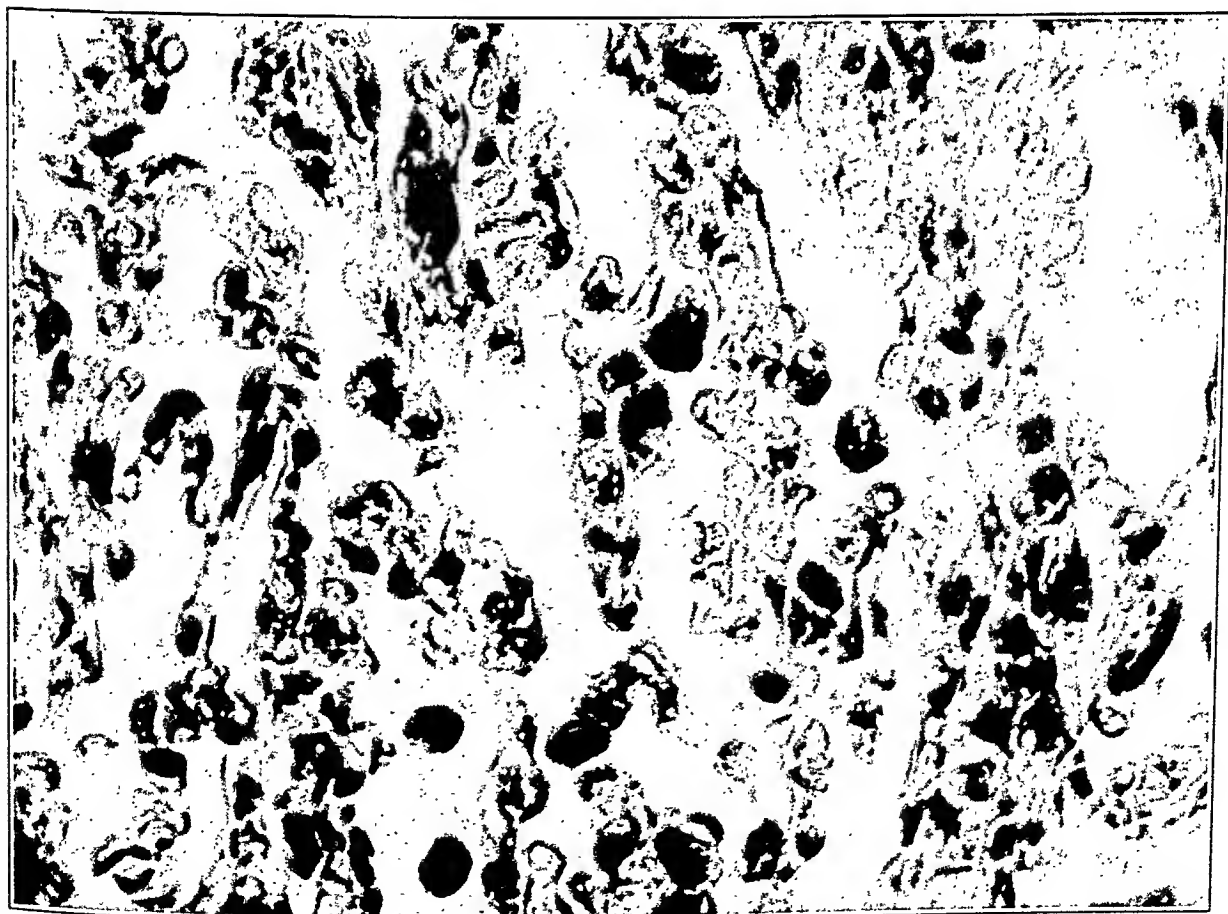
PLATE 94

FIG. 1. A control lung section from a young guinea pig of the type used in this series of experiments. It demonstrates the scarcity of anthracotic pigment phagocytes which are found naturally in the lungs of guinea pigs kept for considerable periods of time in the laboratory pens. $\times 130$.

FIG. 2. A high power lung section of a guinea pig that had received 10 cc. of the standard cell suspension into the jugular vein and was killed 2 days after the injection. Note the numerous carbon-containing mononuclears free in the alveolar spaces. $\times 700$.



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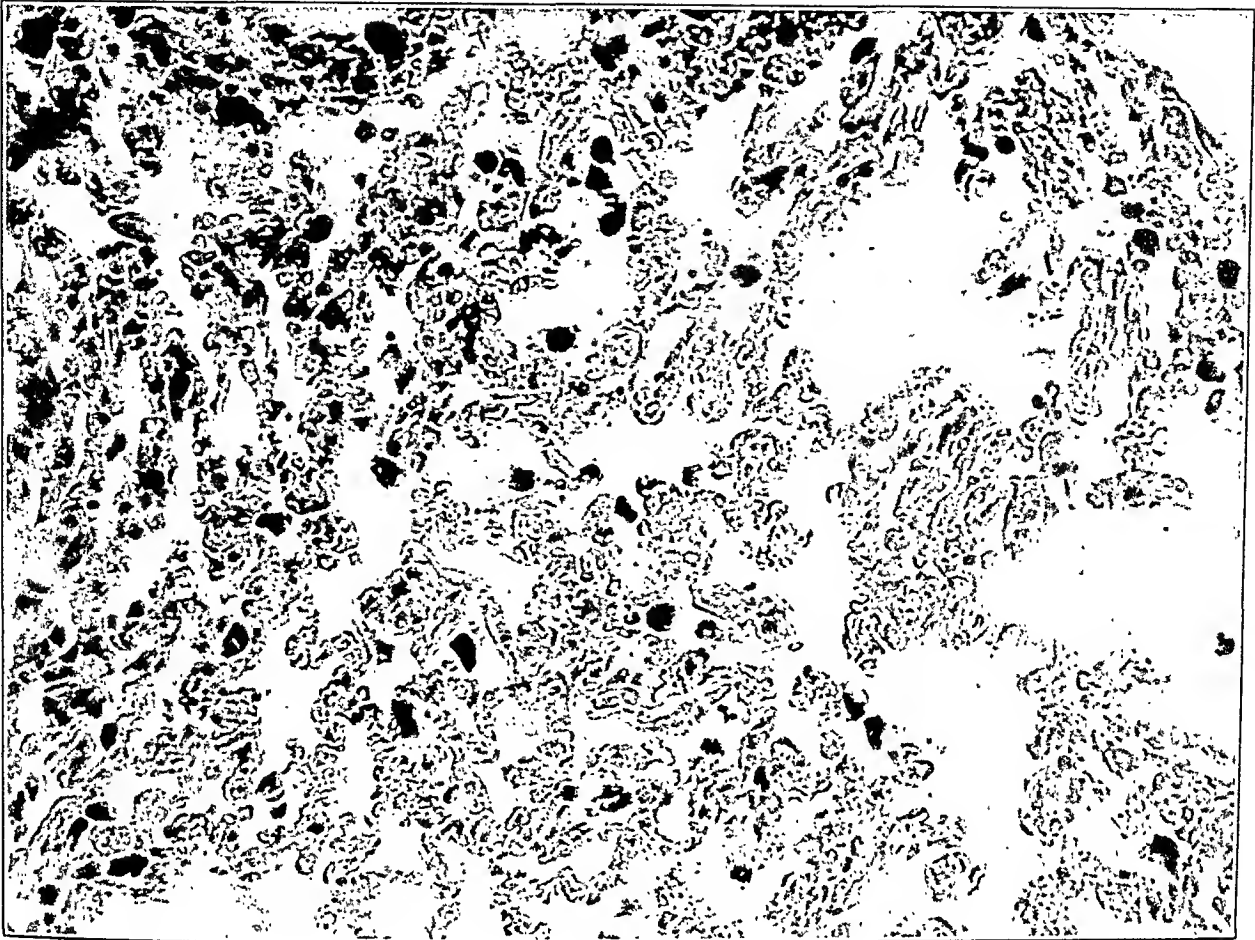


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PLATE 95

FIG. 3. A low power photomicrograph of the lung of a guinea pig that had received an intraportal injection of marked cell suspension 5 days before it was killed. It shows not only the many carbon-containing cells which concentrated in the lung, but also demonstrates the fact that the injected cells were not filtered out in the liver. $\times 200$.

FIG. 4. A high power photomicrograph of the lung of a guinea pig into which a suspension of carmine-containing cells had been injected intracardially 2 days before it was killed. Several injected cells are shown which are so closely applied to the alveolar wall that they simulate alveolar epithelium. The pigment as seen in the section was red in color. $\times 400$.



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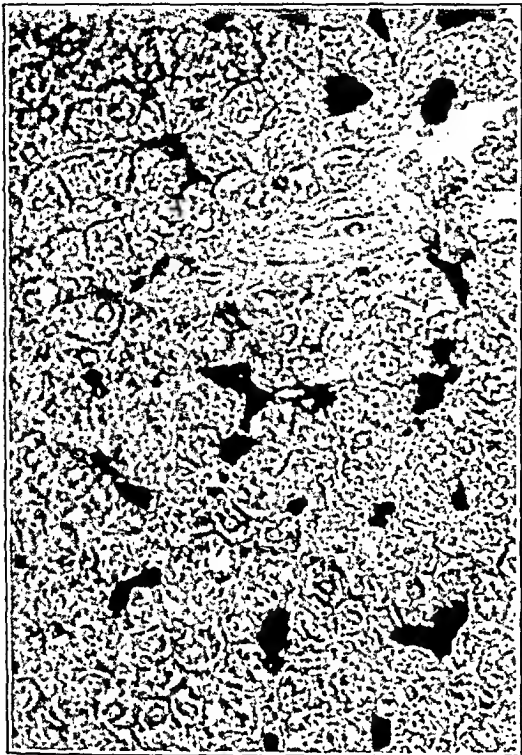
PLATE 96

FIG. 5. The liver of a guinea pig that received dilute India ink intraportally and was killed 3 days later. It demonstrates that when suspensoids, not contained within cells, are injected into the portal vein large quantities of it are retained within the sinusoids, either as embolic masses or within the Kupffer cells. $\times 200$.

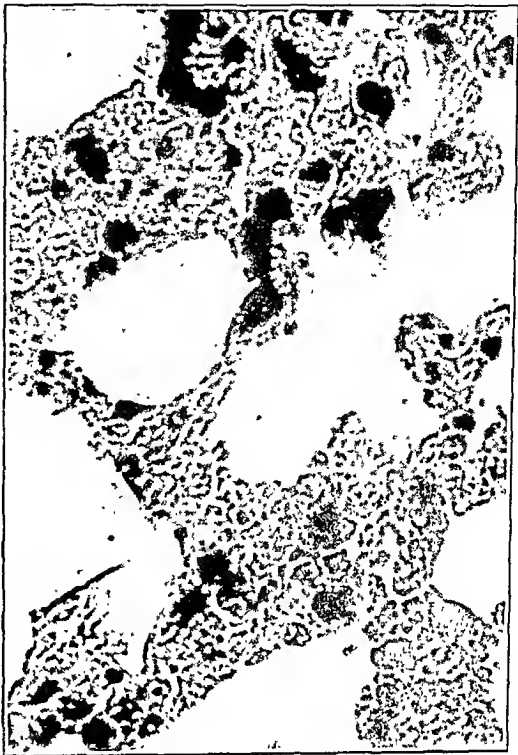
FIG. 6. The lung of a guinea pig that received a large amount of India ink suspension intracardially and survived for approximately 24 hours. It demonstrates that even when massive amounts of suspensoid dye are injected at the most favorable locations, no diffusion into the alveolar spaces occurs. $\times 400$.

FIG. 7. The liver of a guinea pig that had received the cell suspension intraportally 3 days before it was killed. Note that only two small particles of carbon are seen in the entire field, demonstrating the fact that the pigment-bearing cells pass through the capillary beds of the liver to gain the lung with very slight destruction. $\times 200$.

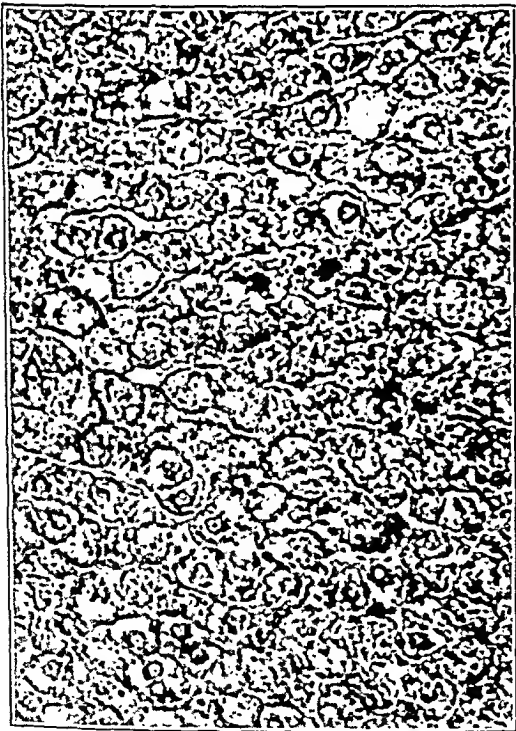
FIG. 8. A high power photomicrograph of the lung of a guinea pig that received a suspension of pigment-bearing cells into the jugular vein 24 hours before it was killed. Large, swollen, carbon-containing cells may be seen lining the alveoli in several locations, but of special interest is the cell in the upper alveolus which is in the process of escaping from the capillary to gain the alveolar space. A large portion of this cell is already applied to the alveolar wall but a portion of it may still be seen within the capillary lumen. $\times 700$.



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CHRONIC PULMONARY ARTERITIS IN SCHISTOSOMIASIS
MANSONI ASSOCIATED WITH RIGHT VENTRICULAR
HYPERTROPHY *

REPORT OF A CASE

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Schistosomiasis is a rare disease in North America. Cutler,¹ reviewing the literature in 1926, was able to collect 22 cases. No additional cases have been reported in North America since that review. The cases appearing in the United States have been acquired in Africa or the West Indies. In the latter country only schistosomiasis mansoni is endemic.

The life history of the parasites has been studied and reported by various investigators. Most recently Faust, Jones and Hoffman² have studied the life cycle and the course of infestation of *Schistosoma mansoni* by the use of experimental mammals, rats, rabbits and monkeys. They find that the lateral-spined eggs, obtained from the stool of patients with the disease, contain ciliated larvae (miracidia). The infective organisms, the cercariae, appear 24 to 35 days later by rupture of the sporocyst. The cercariae, making their way into the animal through the skin, are carried by the blood to the lungs. Thence they migrate passively via the pulmonary venules to the left side of the heart, aorta and mesenteric artery and tend to accumulate in the portal system. From the organs fed by the aorta, they pass again through the lungs and eventually accumulate in the liver. According to Manson,³ about 6 weeks after penetration of the host the trematodes reach maturity, at which time the females lay their eggs in the portal system.

Dew⁴ has studied the lesions produced by the parasite and its ova. The adult female makes its way in the vein against the current. On reaching a point in the vein impeding further progress, the ova are deposited; the spine engages in the wall of the vein, following which

* Received for publication January 7, 1935.

the ovum is forced through into the perivascular tissues. The ovum is first surrounded by leukocytes, among which the eosinophile is conspicuous. Surrounding the leukocytes there is a reaction of the fixed tissue cells and large and small round mononuclear cells are scattered peripherally. The ovum may be engulfed by phagocytic cells or giant cells may be formed around it. The aggregation of eosinophilic cells, lymphocytes and tissue cells around central giant cells constitutes the bilharzial "tubercle." The phagocytosis of the ovum may go on until it is replaced by fibrous tissue, and finally a fibrous nodule results with fragments of the chitinous eggshell remaining to show its true nature.

In *S. mansoni* infections the severest lesions are found under the mucosa of the colon, rectum, and in the liver. Dew⁴ states that in pure *S. mansoni* infections there is an almost complete absence of lung changes, contrasting with the findings in infection with *S. hematobium*. In the latter, pigmentation and patchy fibrosis may be found. However, Hutchison⁵ in his discussion of the pathology of schistosoma infections states that "ova have been discovered in the lungs, brain and kidneys." He used material brought from Egypt. Day⁶ mentions that bilharzial lesions may be found in the lungs of patients who have died with "bilharzial cirrhosis" caused by the *Schistosoma mansoni*.

Vascular lesions in schistosomiasis were first described in 1905 by Letulle⁷ who called attention to lesions of the veins of the intestine in schistosomiasis hematobia. He investigated 1 case of this disease with great thoroughness and described fibrous intimal thickening in the veins of the mucosa, submucosa and muscularis. The thickening was eccentric or concentric and led to considerable diminution in the lumen of the vessel. Nakamura⁸ described lesions in the veins in *Schistosomiasis japonica*. These were of various types. There were embolic lesions consisting of ova lodged in the finest portal branches, around which pseudotubercles with foreign body giant cells developed. He also saw thickening of the walls of the larger portal twigs and thrombus formation. Both he and Benda,⁹ who studied *S. japonica*, found focal intimal thickening in the branches of the portal vein, which consisted of granulomas comprised chiefly of fibroblasts with few lymphocytes and large giant cells of the Langhans's type enclosing ova or their shells. Benda stated he could find no assertions in the literature concerning vascular lesions

caused by *S. mansoni*. Hutchison⁵ asserts that thrombosis of the veins is common, and refers to thrombosis of the femoral, abdominal vena cava and the portal vessels, but does not mention the type of schistosomiasis in which this was observed.

There are two reports of arterial lesions associated with infestation by the trematode. Sorour,¹⁰ from a study of material in Cairo, reported lesions which he considered "exactly similar to the well-known vascular lesions found in syphilitic and arteriosclerotic cases with no bilharzial element." He described lesions in which the presence of the ovum within the lumen excited marked proliferation of the endothelium enclosing the ovum and obliterating the lumen. He noted proliferation of the subendothelial tissue in branches of the pulmonary artery. Bilharzial tubercles were noted within the muscular coat of vessels which revealed an aneurysmal-like vascular projection. He does not state the size or the location of the vessels affected. The type of schistosome parasite associated with these lesions is not recorded. No illustrations are given.

Bey¹¹ described 2 cases of dilatation of the pulmonary artery associated with "fibrosis of the lungs and splenomegalic cirrhosis of the liver" in Egyptian peasants heavily infected with bilharzia, both urinary and intestinal. An autopsy performed in 1 case following sudden death revealed the presence of bilharzial lesions in the liver, intestines, bladder and lungs. The lungs are described as "fibrotic, tough and emphysematous." Microscopically there were "advanced peribronchial and perivascular bilharziomata." "The bilharzia ova were deposited in the adventitia of the blood vessels, and in these last, a generalized endarteritis obliterans was quite manifest." The lesions are not illustrated. The pulmonary artery in gross revealed diffuse dilatation and was studded with raised rubbery patches, which microscopically proved to be early atheromatous lesions. In the description of the heart extreme dilatation of the right side was noted and an "embolus" was found filling one of the main branches of the pulmonary artery; the source of the embolus is not mentioned.

Because of the inadequacy of reports in the literature dealing with vascular involvement in schistosomiasis mansoni, and because a cardiac complication has not hitherto been described, it seemed worth while to record the following case.

REPORT OF CASE

Clinical History: T. C., a 21 year old Porto Rican female, was admitted to the wards of the Third (New York University) Medical Division of Bellevue Hospital, July 31, 1934, with the chief complaint of shortness of breath and swelling of the legs.

The family history was irrelevant. The patient was born in Porto Rico, where she had lived until the age of 9. For the next 12 years she resided in New York. Since the age of 14 she had had palpitation and shortness of breath on exertion, and during that year she was told by her school physician that she had heart disease. In 1931 precordial pain first appeared. In November, 1931, she entered the Metropolitan Hospital complaining of pain in the right lower abdominal quadrant. The records of that hospital reveal no significant physical findings except for the heart which was described as follows: "The apical impulse was forceful; the point of maximal intensity in the fifth interspace within the midclavicular line. There was an apical systolic thrill and a long musical systolic murmur heard all over the precordium and back; the rhythm was regular." The patient was discharged improved, soon after admission, with the clinical diagnosis of acute salpingitis.

In February, 1932, the patient was readmitted to the Metropolitan Hospital for vaginal discharge and pain in the right upper abdominal quadrant of 2 weeks duration. The findings on this admission were similar to those of the previous admission.

She did not come under medical observation again until her present illness, which began 1 month previous to admission to the Bellevue Hospital. She experienced marked increase in shortness of breath and observed swelling of the legs. Following this, swelling of the abdomen was noted, and dyspnea became so marked that she found it necessary to sleep in the semirecumbent position. For 3 days prior to admission she experienced pains in the precordium and in the right chest.

Physical examination revealed an acutely and chronically ill young negress who exhibited marked dyspnea and orthopnea. Because of her race cyanosis was not evaluated. The pupils were equal, regular and reacted to light. Examination of the ears, nose and throat was negative. The veins of the neck were dilated in the recumbent, semirecumbent and upright positions, pulsated and filled from below. There were dullness, diminished breath sounds and an occasional râle at the right base. The point of maximal intensity of the apical impulse was in the fifth interspace outside the midclavicular line. Systolic and diastolic murmurs were present at the apex; P_2 greater than A_2 ; rhythm was regular, gallop audible and the rate was 120. The blood pressure was 100/68. The abdomen was distended with fluid; the liver and spleen were not palpated. There was marked pitting edema of the lower extremities extending to the sacrum. The rectal temperature was 101° F.

Laboratory Examination: Red blood cells 3,700,000 per cmm. Hemoglobin 60 per cent. White blood cells 8600 with 76 per cent polymorphonuclear leukocytes, 4 per cent metamyelocytes II, and 20 per cent lymphocytes. Urine was negative but for the presence of a trace of albumin. Blood culture was sterile. Blood chemistry revealed the following values: non-protein nitrogen 47, uric acid 7.5 mg. per 100 cc.

The patient was rapidly digitalized but grew increasingly dyspneic and died 20 hours after admission.

Clinical Diagnoses: *Cardiac: (a) unknown (rheumatic type, inactive and active); (b) enlarged heart, mitral insufficiency and mitral stenosis; (c) sinus tachycardia; (d) class III (failure at rest).

SIGNIFICANT AUTOPSY FINDINGS

Bellevue Hospital autopsy No. 20856, performed on Aug. 1, 1934, 4 hours after death, revealed the body to be that of a young negress about 155 cm. in length and weighing about 120 pounds. There were numerous pinhead macular-papular lesions, dark red in color, over the anterior thoracic region. There was marked edema of the legs, thighs, anterior abdominal wall and sacrum. The nailbeds were dusky blue, and there was no clubbing. There were wedge-shaped hemorrhages in the sclera of both eyes on either side of the pupil.

On section the subcutaneous fat was moderate in amount, the muscle tissues well developed and deep red in color. There were about 2000 cc. of clear amber fluid in the peritoneal cavity. The peritoneum was smooth and glistening but the subserosal tissues were markedly edematous.

On opening the chest the precordium was seen to be markedly enlarged, chiefly to the right, compressing and displacing the right lung. Both pleural cavities were dry and there were no adhesions between the lungs and the chest wall.

Heart: On opening the pericardial sac about 300 cc. of a clear, straw-colored fluid were encountered. There were no adhesions between the visceral and parietal layers of the pericardium. The anterior aspect of the heart was occupied chiefly by the right ventricle. The right auricle was found to be dilated. The orifice and the leaflets of the tricuspid valve were normal. The right ventricle was found to be dilated and its walls hypertrophied. The maximum thickness of the wall of the right ventricle was 8 mm. (Fig. 1). The pulmonary artery was dilated, measuring 7 cm., while the aortic ring measured only 5 cm. The pulmonic cusps were thin, transparent and freely movable. A few circumscribed, irregular, raised yellowish plaques were visible in the various sized branches of the pulmonary artery. The left side of the heart appeared dwarfed by comparison with the

* Diagnosis conforms to nomenclature of American Heart Association.

right. The maximum width of the wall of the left ventricle was 10 mm. The mitral and aortic valves were normal. The aorta and coronary vessels presented no notable characteristics. The heart weighed 360 gm.

Lungs: Both lower lobes were distinctly heavier than the upper and middle lobes and of a deep red color, contrasting with the pinkish gray color of the latter. On section a red frothy fluid exuded from the lower lobes whereas the upper lobes were but slightly moist. The small arteries appeared to project from the surface and felt thickened. The trachea and bronchi contained a slight amount of mucoid material.

Liver: The liver appeared small, weighing 960 gm. Its surface was nodular, the nodules varying in diameter from 2 to 10 mm. The color was a yellowish brown with irregular patches of purple. The organ was of increased firmness and cut with increased resistance. The essential pattern of the cut surface was that of a mosaic, the units of which were round or oval in outline and varied from 1 to 2 cm. in diameter (Fig. 2). The periphery of these units was formed by an irregular dark red border 1 to 3 mm. in width. In the center was a vessel cut in cross-section, 2 to 3 mm. in diameter and surrounded by a small amount of connective tissue. The intervening tissue revealed no distinctive features. The portal vein was empty and its walls appeared normal. No adult worms were found.*

The gall-bladder and biliary passages were normal.

Spleen: The organ was about thrice its normal size, purplish red in color with a thin smooth capsule. On section it presented a smooth surface, dark homogeneously red and firm. The follicular markings were not visible. The splenic vein was normal.

Gastro-Intestinal Tract: In the lower third of the esophagus several dilated veins were readily made out and two bleeding points about 4 cm. above the cardia were seen. The stomach was moderately dilated and contained about a half liter of coffee-ground material and several small blood clots. Thin, blackish brown material was also seen in the small intestine. The mucosa and intestinal wall, including that of the rectum, appeared normal. The hemorrhoidal veins were not opened.*

The pancreas, adrenals, kidneys, ureters and bladder were normal.

* On gross examination the diagnosis of schistosomiasis was not suspected.

Genitalia: There were two hemorrhagic cysts on the surface of both ovaries, each about the size of a pea. The tubes and uterus were normal.

Head: Not done.

MICROSCOPIC EXAMINATION

Sections were fixed in formalin, stained with hematoxylin and eosin, Weigert's elastic tissue method, Van Gieson, and occasional sections were stained with the silver impregnation method of Foot and Foot.

The diagnosis of schistosomiasis was established by the presence of ova of this trematode in many organs. They were found in large numbers in fixed tissue sections in the lungs, myocardium, liver, pancreas and kidney. The most typical ovum seen in fixed tissue sections appears as an oval eosinophilic mass 35 to 70 μ , containing irregularly scattered, coarse, basophilic chromatinic material, and separated by a clear space from the chitinous shell. The latter is irregularly wavy, continuous or interrupted, single or appearing reduplicated, and has a clear, refractile, straw-colored appearance (Fig. 3). Occasionally a laterally placed spine is seen. The lesions provoked by the presence of these ova are to be described in the individual organs in which they occurred.

Liver: In sections the structural unit appears to be that consisting of a centrally placed, massively thickened portal radicle surrounded by liver lobules and outlined peripherally by an area of hemorrhage and necrosis of the liver trabeculae. The central area consists of rather dense collagen which displays radiating prolongations into the surrounding tissue. The periportal tissue is markedly increased in amount. There are several thin-walled veins and small arteries cut in cross-section, the walls of the latter being somewhat thickened. Medium size bile ducts are included. The connective tissue reveals foci of cells. Many such foci consist of a central core of an ovum surrounded by lymphocytes, eosinophilic leukocytes and plasma cells. Other foci show dense accumulation of lymphocytes with few eosinophiles and plasma cells and no ova. The inflammatory reaction is limited, chiefly, to the areas surrounding the ova; there is no reaction in the tissue surrounding the vessels or ducts. Ova are numerous, 1 to 2 are present in each medium power field.

The surrounding parenchyma has retained its identifying architecture, with liver trabeculae and portal radicles, and occasional central veins. The trabeculae are for the most part well preserved in outline, but the cells stain faintly with eosin. Scattered irregularly are large areas of very poorly staining cords showing granular disintegration of the cytoplasm with disappearance of the nuclei and at times complete disintegration of the cords. The Kupffer cells contain brown pigment which does not give the iron reaction. The portal radicles encountered in these areas reveal no notable change other than the presence of a degenerated ovum and inflammatory cells. The more peripheral hemorrhagic zone consists of broad, irregular areas of intense hemorrhage with obliteration of any recognizable liver trabeculae. Between these areas of hemorrhage are those in which hemorrhage is absent, liver trabeculae have disappeared and there remains but the reticular framework of the organ with many proliferating bile ducts.

Spleen: There is marked diffuse congestion of the pulp and a fine increase in the connective tissue framework. There are numerous polymorphonuclear leukocytes and a few eosinophiles visible in the pulp. No ova are seen.

Pancreas: Foci of ova surrounded by inflammatory cells similar to those in the liver are seen. In some areas the interlobular connective tissue is diffusely infiltrated by cells among which the eosinophilic leukocyte predominates. The ova are found intra-lobularly as well as in the interlobular tissue. There are no areas of necrosis, no increase in the stroma of the gland, and the vessels appear normal.

Kidney: Many ova are seen in the cortex, some of which are recognizable only by the chitinous shell. They are surrounded by numerous lymphocytes and eosinophilic polymorphonuclear cells. They occupy an interstitial position without any particular relation to the blood vessels; the latter appear normal. The glomeruli and tubules are normal.

Myocardium (Right Ventricle): There is evidence of hypertrophy of the muscle nuclei and fibers and focal interstitial fibrosis. An occasional ovum surrounded by a few inflammatory cells is seen. The left ventricle reveals the presence of a few ova and inflammatory cells. The blood vessels appear normal.

Aorta: The vessel appears normal.

Pulmonary Artery: The intima is slightly thickened and presents atheromatous plaques.

Lung: The pulmonary parenchyma appears for the most part normal. There are irregular areas of marked congestion, some of focal alveolar hemorrhage and patchy collapse. The striking finding is that of granulomatous lesions within or related to the blood vessels. Some of these granulomas appear clearly to be in the periadventitial connective tissue and the vessel may appear normal. The granuloma consists of one or more ova, which may contain a foreign body giant cell. These ova are surrounded by young fibrillar tissue (argentophile fibers) in which are spindle and oval fibroblasts, lymphocytes and eosinophiles, and numerous, small endothelial-lined channels. But more frequent than these extravascular lesions are those that appear more intimately related to the blood vessels. Figure 4*b* illustrates one such lesion. It appears as a vascularized granuloma in the interstitial tissue related to a bronchus. In the hematoxylin and eosin sections it is chiefly its relation to a bronchus which suggests that it is an artery. Examination of the Weigert elastic sections strengthens this impression, for a wavy elastic membrane outlines the structure peripherally. Indeed, it is possible to see fragments of internal and external elastica with intervening muscle in the periphery of this granuloma. But in order to ascertain with greater certainty the relation of such structures to the pulmonary arteries, serial sections were studied. Figure 4*a* shows a small artery with thickened intima, measuring 200 μ in diameter, cut in somewhat longitudinal section. Figure 4*b*, which is 55 μ distal, reveals the same vessel, considerably dilated, the lumen obliterated and replaced by an ovum surrounded by young granulation tissue with numerous endothelial-lined channels. The media is represented by internal and external elastica with intervening muscle in about a fourth of its circumference; fragmented elastic fibers are seen peripherally for part of its circumference. A mantle of inflammatory cells surrounds the structure and infiltrates the wall of the adjacent bronchus. The cells are chiefly of the lymphocytic type; few eosinophiles are seen. Figure 4*c*, 110 μ distal to the preceding section, is apparently distal to the granulomatous lesion; the lumen of the artery is patent, its wall intact; the diameter of the vessel is half that in the preceding section.

Many small arteries are seen showing marked concentric intimal thickening with diminution of the lumen. The intimal thickening

consists of young connective tissue, avascular and containing but few fibroblasts. Medial hypertrophy appears to be present. Several such vessels were traced serially and their association with a schistosomal lesion was demonstrated. Figure 5a illustrates another lesion of this type in a small artery $250\ \mu$ in diameter. As this vessel is followed distally, a granuloma can be seen to form between the external elastic membrane and the adventitia. The elastica, in further sections, becomes reduplicated, then disrupted and the vessel assumes an aneurysmal dilatation with the lumen almost completely occluded by proliferating tissue continuous with the intramural granuloma. Figures 5b and 5c, $195\ \mu$ distal to the preceding section, illustrate these changes. Comparison of Figures 4a, 4b and 4c reveals the striking dilatation of the vessel in its distal course. Three ova are to be found in the course of this vessel, two of which occupy an intramural position.

Final Pathological Diagnoses: *Lungs:* Chronic pulmonary arteritis (*Schistosoma mansoni*), intimal hyperplasia and focal medial hypertrophy, congestion, focal alveolar hemorrhage, patchy collapse. *Heart:* hypertrophy and dilatation, chiefly right ventricular, miliary schistosomal granulomas, dilatation and atheroma of the pulmonary artery. *Liver:* chronic diffuse granulomatous hepatitis (*S. mansoni*), chronic passive congestion, focal hemorrhage, focal necrosis. *Spleen:* chronic passive congestion. *Pancreas:* miliary schistosomal granulomas. *Kidneys:* miliary schistosomal granulomas.

DISCUSSION

While there are many features for discussion in this case, including the widespread dissemination of the ova and the peculiar hepatic lesion, we shall confine ourselves to a consideration of the findings in the pulmonary vessels. The demonstration of ova in constant intimate relation to the vascular lesions in the lungs leaves no doubt as to the parasitic etiology. The presence of the ovum within the lumen or the wall of the vessel has been followed by complex changes leading to the obliteration of the lumen by a richly vascularized tissue. The absence of early lesions in the pulmonary vessels does not permit reconstruction of these lesions from their inception. The rich vascularization of the tissue surrounding the ovum and occluding the lumen might suggest this lesion to be the result of thrombosis with

organization and canalization. But the severe medial changes certainly must be attributed to an injury of a different nature. The absence of collagen and the presence of proliferating endothelium, forming new channels, suggest that these lesions are still young and blood pigment should be present nearby if thrombosis had occurred. Moreover, the same proliferation of endothelial-lined channels is seen in extravascular granulomas in the lung. It appears more likely that the intimal and endothelial proliferation represents a response to the presence of the foreign body.

The medial injury might be effected from within by an expanding proliferative lesion, or from without by the presence of an inflammatory and proliferative reaction provoked by the intramural ova. It appears likely that the intimal thickening and medial hypertrophy of the small arteries proximal to the granulomatous lesion represents a response to the obstruction. The atheroma of the pulmonary artery similarly is to be interpreted as a response to the increased intravascular tension which had its origin in the obstructive arterial lesion.

Thus, the vascular lesions may be separated into two groups: those directly attributable to the ova, and those secondary to the increase in intra-arterial tension. The former may be considered a specific arteritis, characterized by an obliterating endarterial proliferation with marked injury to the media leading to dilatation of the vessel. The latter may be considered as representing a non-specific vascular change of an arteriosclerotic and atheromatous nature.

The presence of hypertrophy of the right ventricle and evidence of congestive heart failure are obviously related to the lesions of the small pulmonary arteries.

The case presented thus represents one in which the cardiac changes have occurred secondary to pulmonary arterial disease. In this respect it is to be compared with those cases of pulmonary heart disease associated with arterial and arteriolar lesions of the lungs and described under the terms of primary sclerosis of the pulmonary artery (Bacon and Apfelbach¹²), die primäre Pulmonalsklerose (Steinberg,¹³ Höra¹⁴), and Arteriopathia pulmonalis (Bredt¹⁵). There is little known concerning the etiology of these cases. Arriaga¹⁶ believed some cases to be due to syphilis. Steinberg¹³ described a case in which the vascular lesions were interpreted as

pulmonary arteriolar sclerosis secondary to "essential pulmonary hypertension." Bacon and Apfelbach¹² reported a case in which the symptoms appeared after influenza, and they suggested a relation of the pulmonary vascular lesions to the latter disease. Rössle¹⁷ considered the cases described by Bredt as representing a non-specific allergic arteritis related to rheumatic fever and assigns to them the term "rheumatoid vascular disease." Höra¹⁴ interpreted the lesions in his case as evidence of chronic sepsis. In all these cases morphological comparisons have served as the basis for the interpretation of their etiology. In our case of schistosomiasis mansoni the presence of the etiological agent in the inflammatory lesions of the smaller pulmonary arteries has been demonstrated.

SUMMARY

A case of schistosomiasis mansoni in a young Porto Rican dying in congestive heart failure has been recorded. Hypertrophy of the right ventricle, dilatation of the pulmonary artery and lesions of the small pulmonary arteries have been described, and it has been demonstrated that the latter represent a form of specific arterial disease caused by the presence of ova of *Schistosoma mansoni*.

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DESCRIPTION OF PLATES

PLATE 97

- FIG. 1. An anterior view of the opened heart revealing the dilated pulmonary conus and marked hypertrophy of the right ventricular myocardium.
- FIG. 2. A view of the cut surface of the liver showing the peculiar lobulations and zones of hemorrhage.
- FIG. 3. Photomicrograph of a section of the pancreas showing two ova in a granulomatous nodule. Hematoxylin and eosin stain. $\times 350$.



PLATE 98

FIG. 4a. Photomicrograph of a section of the lung showing a small pulmonary artery with thickened intima and beneath it a partially collapsed normal bronchus. Hematoxylin and eosin stain. $\times 110$.

FIG. 4b. Same vessel $55\ \mu$ distal to preceding section. Note the presence of an ovum within the granulomatous arterial lesion, and a segment of the media with internal and external elastic membranes. Weigert's elastic stain. $\times 140$.

FIG. 4c. Same vessel $110\ \mu$ distal to preceding section. The section is distal to the granulomatous lesion and dilatation is no longer evident. Weigert's elastic stain. $\times 95$.

FIG. 5a. Photomicrograph of a section of another small pulmonary artery revealing marked intimal thickening and medial hypertrophy. A portion of the wall of the adjacent bronchus can be seen at the bottom of this and succeeding photographs. Weigert's elastic stain. $\times 180$.

FIG. 5b. Same vessel, $195\ \mu$ distal to the preceding section, revealing marked dilatation, replacement of the walls and occlusion of the lumen by vascularized granulomatous tissue containing several ova. Hematoxylin and eosin stain. $\times 140$.

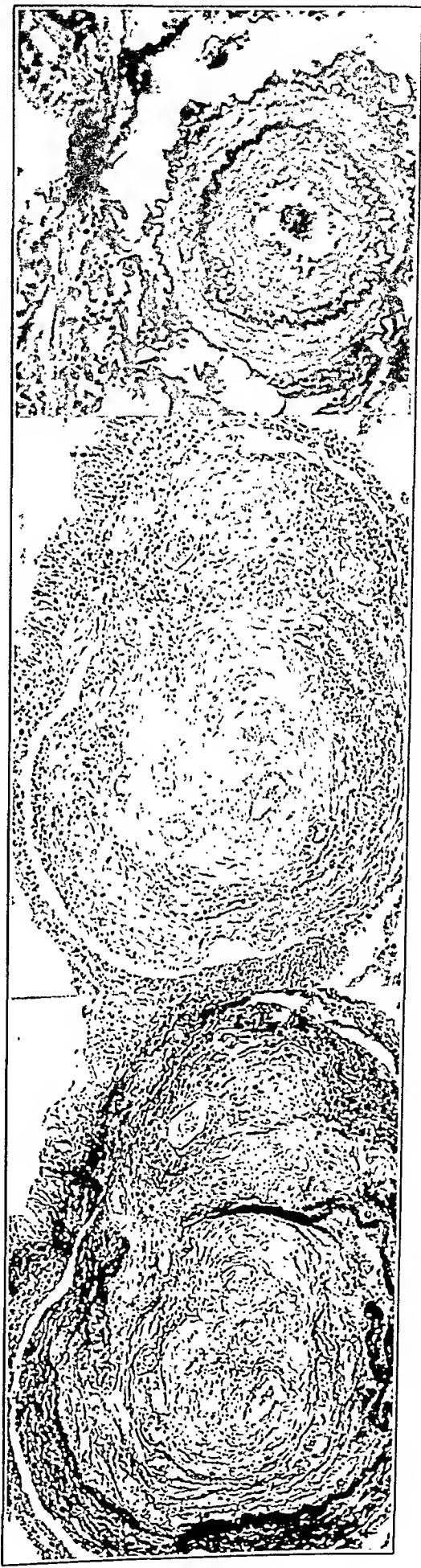
FIG. 5c. Same section stained with Weigert's elastic tissue stain. Note the disruption of the elastic membrane. $\times 140$.



4a

4b

4c



5a

5b

5c

EARLY CARDIAC INFARCTION CAUSED BY AN EMBOLUS OF CASEOUS TUBERCULOUS MATERIAL *

REPORT OF A CASE

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Gouley *et al.*,¹ state in their recent report on tuberculosis of the myocardium that "while tuberculous pericarditis is not uncommon, tuberculous involvement of the myocardium is decidedly rare." The author presents in this connection a tuberculous involvement of the heart which he has been unable to find referred to in the literature, namely, infarction of the heart muscle due to an embolus of caseous tuberculous material.

The case was that of a white male aged 40 years whose death was entirely unexpected. The man had been ill for over 6 months but was not considered ill enough to be confined to bed during this time. He was an ambulatory patient up to the day of his death. A diagnosis of pulmonary tuberculosis had been made but this condition was not considered of sufficient severity to account for the sudden death. Because of the circumstances of death a medicolegal autopsy was requested.

The autopsy revealed extensive bilateral pulmonary tuberculosis with a single small cavity present in the upper right lobe. There was also a generalized miliary tuberculosis. The finding of main interest was the presence of a recent infarction of the lateral and upper portion of the left ventricle. The infarcted area measured 3 cm. in length, 1.5 cm. in breadth and extended to within a short distance of the endocardium. In the gross it presented the usual appearance of an infarct of not more than a few days duration. The epicardial vessels over the area were congested and there was a zone of congestion about the gray, swollen, infarcted muscle tissue.

The heart presented no pathological lesions other than the infarct. The valves and endocardium appeared normal. The coronary vessels, as far as could be determined on gross dissection, showed no

* Received for publication March 15, 1935.

atherosclerosis and no gross evidence of thrombosis or embolism. The pericardial cavity contained about 50 cc. of a light yellowish, slightly turbid fluid.

The nature of the infarction was not suspected until a microscopic study was made. The infarcted muscle cells were disintegrating. There was considerable edema and abundant cellular infiltration, especially around the periphery of the lesion. The cells were largely monocytes and lymphocytes with, in places, a generous sprinkling of neutrophils. There was no evidence of tubercle formation or Langhans's giant cells. In other words, the histopathological picture of the infarcted heart muscle was that of a non-infectious lesion.

In the epicardium a small branch of the coronary artery lying over the infarcted area was found to be plugged by a bit of caseous material in which tubercle bacilli were easily found. Even here there was no evidence of tubercles, giant cells or even acute inflammation. The wall of the artery appeared to be normal. Very careful search failed to reveal any tubercle bacilli within the area of infarction.

We have here, then, in an individual with generalized miliary tuberculosis, an early, acute cardiac infarct caused by a bit of caseous material from a pulmonary tuberculous lesion. As a tuberculous embolus it entered the circulation in the pulmonary vein and lodged in a small branch of the left coronary artery.

COMMENT

It is becoming generally recognized that hematogenous dissemination of tubercle bacilli is the predominant method by which tuberculous foci are established outside of the respiratory and gastrointestinal tissues. This of course excepts the lymph nodes, such as the cervical, peribronchial and mesenteric, which usually become involved through lymph drainage from foci of infection in the tonsil, lung or intestine.

From the literature it would seem that there is a consensus of opinion that tuberculous involvement of the heart and its membranes occurs through a reversal of lymph flow from mediastinal tuberculous nodes. Thus, Gouley *et al.*,¹ give the impression that 5 out of their 6 cases of myocardial tuberculosis are thus caused. Kast² reports a case in which he considers the heart involvement was brought about through the rupture of a caseous node into the

pericardium. It appears that the direct extension, by rupture, of tuberculous disease into the pericardial sac might account for an occasional case of tuberculous pericarditis. It does not appear logical, however, that reversal of lymph flow from tuberculous lymph nodes in the mediastinum would account for the majority of tuberculous involvements of the epicardium and myocardium.

Perhaps one of the reasons why the hematogenous nature of tuberculous foci in the heart or the pericardium is not appreciated is that the bit of tuberculous material is so small that it is extremely difficult to identify within the lumen of a capillary around which develops the larger lesion. Another reason for failure to appreciate the hematogenous nature of the lesion is that by the time the case has come to autopsy the disease process has been in existence so long that it is impossible to determine the site and nature of the original lesion.

The cardiac involvement reported in this article is of real significance relative to hematogenous dissemination of tuberculous material. First, the lesion is of such recent occurrence that the nature and location of the tuberculous material can definitely be determined. Second, the caseous embolus, having demonstrable tubercle bacilli, was within a coronary artery. And third, the embolus was of sufficient size to cause a typical infarct of considerable size in the left ventricular wall.

SUMMARY

A case of cardiac infarction due to a caseous tuberculous embolus within a branch of the left coronary artery is reported.

NOTE: The material for this report was made available through Dr. A. W. Johnson, Coroner for Saratoga County, N. Y.

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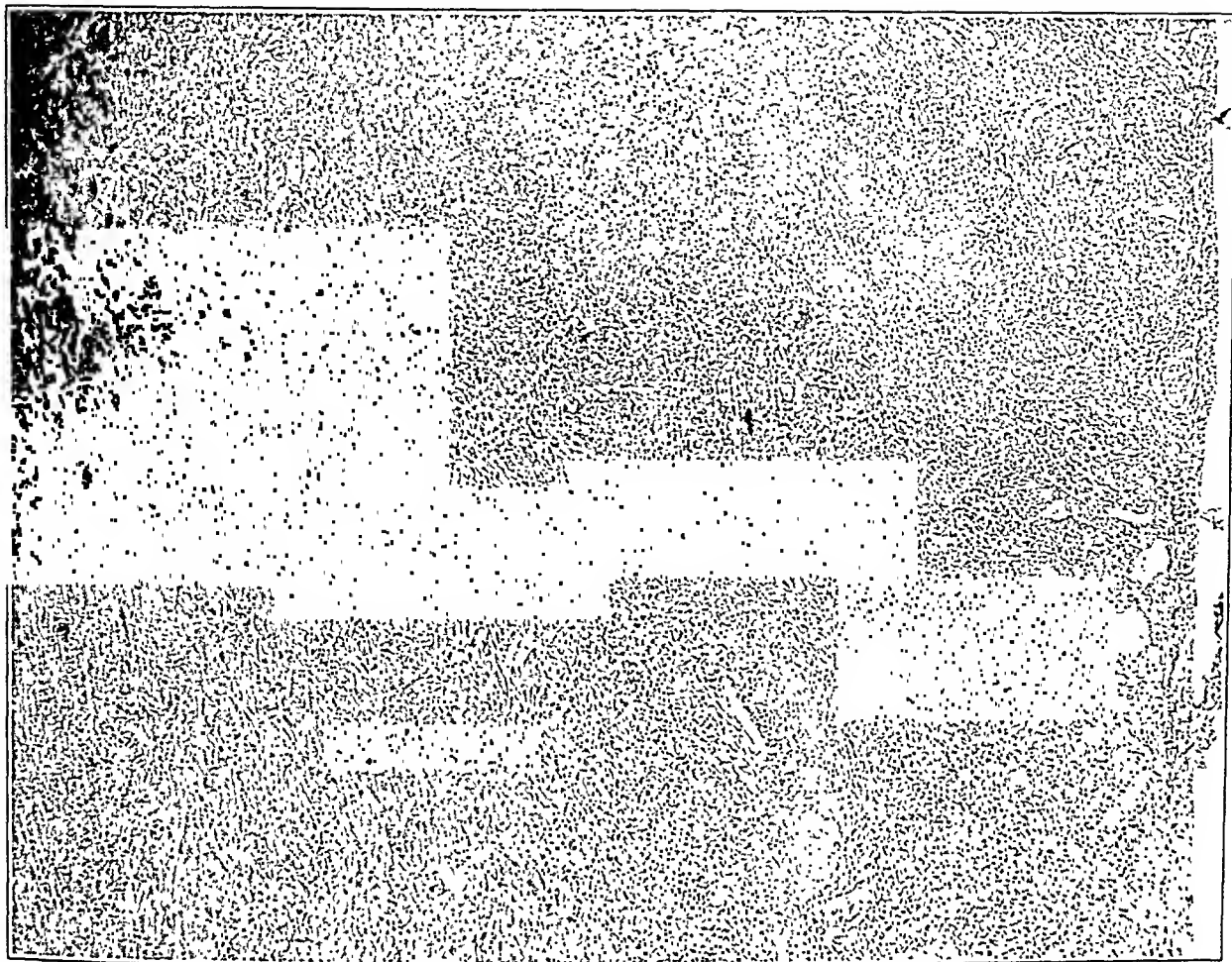
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DESCRIPTION OF PLATE

PLATE 99

FIG. 1. An area of the infarcted heart muscle showing extensive cellular infiltration. $\times 50$.

FIG. 2. Section showing caseous embolus within the coronary artery. $\times 300$.



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THE AMERICAN JOURNAL OF PATHOLOGY

VOLUME XI

SEPTEMBER, 1935

NUMBER 5

LESIONS OF THE LEFT AURICLE IN RHEUMATIC FEVER *

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The influence of the nature and topography of tissues on the character of the resultant inflammatory process is better exemplified by rheumatic fever than by most other chronic diseases. Thus, for example, characteristic individual differences are found in the rheumatic lesions of the myocardium, valve rings, valve substance, pericardium, subcutaneous tissues, tendinous insertions and blood vessels, even though some of these may possess structural or cellular peculiarities in common. This relation between inflammatory process and tissue structure is strikingly shown in the left auricular endocardial lesions.

Rather vague and sketchy references to left auricular lesions were made by Corvisart,¹ Burns,² Baillie,³ Wells,⁴ Laennec,⁵ Hope⁶ and Bouillaud.⁷ From these it is often difficult to determine whether the underlying cause was rheumatic fever, bacterial endocarditis or auricular thrombosis.

In 1898 Claude and Levaditi⁸ gave a gross and microscopic description of what appears to have been an old rheumatic, left auricular endocardial lesion with calcification. They noted distended capillaries, inflammatory cells and obliterative vascular changes near the ulcerated (calcific?) surface. Bacteriological cultures were negative. The case described by Cheadle and Lees⁹ in the same year was undoubtedly one of bacterial endocarditis. This suggestion was made by Poynton, who performed the autopsy.

Huchard's¹⁰ monograph (1903) contains two excellent drawings of

* Aided by grants from the Lucius N. Littauer and Walter W. Naumburg Funds.
Received for publication May 12, 1935.

typical rheumatic auricular lesions illustrating some of the gross and microscopic features of this process. The text description, however, is very vague. In 1914 Harper ¹¹ reported 9 cases of rheumatic infection in childhood. In 1 case the "endocardium of the left auricle was found to be thickened and wavy. This was considered evidence of a former endocarditis." In 1920 Hertel ¹² reported 10 cases of "parietal endocarditis." One of these was a "recurrent endocarditis of the mitral valve." Between the anterior and posterior cusps she observed large deposits, partly warty, partly polypoid, hanging in the heart cavity. These vegetations extended up the left auricular endocardium. An excellent histological description is given of these lesions which Hertel believed were a contiguity process from the mitral valve. However, although the description of this microscopic auricular lesion closely resembles in certain respects the true rheumatic process, the character of the gross lesions and some of the microscopic features suggest the possibility of at least a superimposed bacterial process, in spite of the reported negative bacteriological findings.

The study of this very interesting lesion took on new significance after the publication of the reports by MacCallum (1924-1925).¹³ It is to the great credit of this author that he clearly recognized the essentially rheumatic nature of the left auricular process, gave an excellent description of its gross and microscopic features and, because of his extensive experience in the pathology of rheumatic fever, presented his findings on material which can hardly be subject to question. The most important points brought out by MacCallum were the infiltration of the endocardium and subendocardium with inflammatory cells, the banded appearance of the Aschoff bodies in the endocardium, the presence of necrotic bands of collagen, the edema, the distortion of the elastic lamellae and the extension of the process through the auricular myocardium to the pericardial mantle.

Soon after the publication of the first of these papers, Stewart and Branch ¹⁴ reported a case of rheumatic auricular endocarditis with calcification. The endocardium of the left auricle showed fibrous thickening with irregularity of the surface. The presence of adhesive pericarditis and myocardial Aschoff bodies, together with the clinical history, led the authors to conclude that the auricular lesion was of a rheumatic nature.

In 1926 VonGlahn¹⁵ added materially to our knowledge of this lesion by a description of the auricular changes found in 9 out of 31 cases of rheumatic valvular disease. While no attempt was made to segregate the cases with respect to acuity or chronicity of the rheumatic process in the entire heart, a very careful and detailed description was given of the gross lesions and microscopic findings. In this paper VonGlahn was able to confirm MacCallum's findings and noted in addition the presence of large and small mononuclear cells, as well as polymorphonuclear leukocytes, oriented at right angles to the endocardial wall. He observed fibrin on the surface of some of these lesions and, at times, verrucous deposits. Considerable quantities of fibrin were present in the subendocardium. Capillaries were seen penetrating the endocardial lesion but never beyond the middle portion. Healing took place by means of cells resembling fibroblasts which arranged themselves perpendicularly to the surface in the superficial part of the endocardium, especially between the main connective tissue layer and the endothelium. Later on, elastic fringes and often calcium deposits were seen in these healing and healed areas. Further healing took place by the disappearance of the cellular accumulations.

These descriptions were subsequently amplified by Pappenheimer and VonGlahn,¹⁶ and by VonGlahn and Pappenheimer.¹⁷ In the latter report reference is made to the finding of 3 cases with lesions in the right auricle. Since the publication of these papers several new cases have been described by Shaw,¹⁸ Perla and Deutch,¹⁹ and by Klinge,²⁰ largely confirming the previously reported observations.

On reviewing the findings reported by the above mentioned authors, it appeared desirable to investigate these lesions on a much larger series of material, laying especial emphasis on the correlation between the gross and histological findings in the left auricle and the clinical course of the disease. This report will concern itself with a study of 87 rheumatic cases. Sixty-seven of these were active and showed Aschoff bodies in the myocardium, and 20 showed chronic valvular disease of the rheumatic variety but without evidence of activity either clinically or pathologically and with no demonstrable Aschoff bodies in the myocardium. The grouping as to activity and inactivity was based on the criteria outlined by Rothschild, Kugel and Gross.²¹ Particular care was taken to avoid material which in any way indicated the possibility of a coexisting bacterial endocar-

ditis. A careful study of the clinical records and pathological specimens made it possible to divide the material into the following groups:

GROUP 1. Active cases where death took place in the first attack or where one preceding attack occurred within 1 year of the fatal outcome.

GROUP 2. Active cases in which one previous attack occurred at least 2 years previous to the fatal outcome.

GROUP 3. Active cases with repeated attacks, death occurring during an acute recurrence.

GROUP 4. Active cases where death was caused by decompensation without clinical evidence of a final recurrence. Some of these cases had no previous history of rheumatic fever.

GROUP 5. Inactive cases of chronic valvular disease of the typical rheumatic variety.

The technical methods used were essentially the same as those previously described by Gross and Ehrlich.²² The findings here reported are based on a study of the routine left auricle sections (L.A.) and the auricular portions of the routine mitral valve posterior sections (M.V.P.) obtained in the standardized procedure suggested by Gross, Antopol and Sacks.²³

Before describing the findings in this material it is advisable to present very briefly the histological characteristics of the normal left auricular endocardium, with special reference to the age period changes. It will be found that among the changes produced by rheumatic fever in the left auricular endocardium there occur alterations in the relations of the connective tissue, smooth muscle and elastic tissue components. Since alterations also occur as the result of age, it is necessary to have a clear-cut conception of these processes in order to differentiate the changes that are of normal evolutionary origin from those that may be due to the rheumatic process. This is particularly true for the findings in the inactive cases.

Descriptions of the histology of the left auricular endocardium have been reported by Königer,²⁴ Nagayo,²⁵ Miller and Perkins,²⁶ and by Perkins and Miller.²⁷ The descriptions by Königer and Nagayo appear to be unnecessarily complex and too rigid to fit the not inconsiderable variations found in the normal, human, left auricular endocardium. In the reports by Miller and Perkins, and Perkins and Miller, the age period changes in the left auricular endocardium

are described in a 2 day old infant, a 35 year old individual and an 85 year old individual.

In order to obtain a more plastic impression of the age period changes in a fairly representative number of cases, 50 normal hearts were chosen for study. These specimens were carefully selected to rule out such conditions as may be expected to affect the cardiovascular system. An examination of this material reveals the following histological and topographical features of the left auricular endocardium and their changes from birth to the eighth decade of life.

HISTOLOGY AND AGE PERIOD CHANGES OF THE LEFT AURICULAR ENDOCARDIUM

The fibro-musculo-elastic membrane lying internally to the left auricular myocardium may be divided into two layers, *i.e.* the endocardium proper and the subendocardium (Fig. 1). The former consists at birth of somewhat closely packed, anastomosing delicate sheets of elastic tissue separated by collagen fibrils. The elastic membranes lie parallel to one another and to the lumen of the auricle, running for the most part transversely to the axis of the blood stream flow. Dividing this endocardial layer arbitrarily into three equal zones, *viz.* inner third, middle third and outer third, it may be said that there are no conspicuous concentrations of the elastic tissue in any of the zones but that the outermost one occasionally contains a few smooth muscle fibers with their axis generally parallel to the elastic lamellae. Apart from these, the endocardium shows a large number of fibroblasts with fairly abundant cytoplasm and somewhat vesicular rounded nuclei. The cells are embedded in a mucinoid, more or less basophilic matrix. Within a few months after birth the cytoplasm disappears and the nuclei become denser and somewhat elongated. Scattered, large mononuclear cells, occasional lymphocytes and rare polymorphonuclear leukocytes may also be seen. These cells occur rather infrequently until about the fourth decade of life when they practically disappear from the normal endocardium. The number of fibroblastic nuclei appears to fall off sharply within the first year of life. They generally occupy the middle and outer thirds of the endocardium. From the third decade on they become very sparse. No blood vessels are seen in the normal endocardium.

The endocardium is covered by flat endothelial cells. Between these and the outermost elastic lamellae there are found a few scattered cells apparently derived from the primitive mesenchymal layer which, because of their multipotential properties under certain inflammatory conditions, will be referred to as the "mesenchymal layer." It is to be noted that this layer is most inconspicuous in the normal left auricle.

Immediately external to the endocardium proper, and intimately bound up to it, lies the subendocardial layer. This consists of larger and denser collagenous masses separated by interlacing elastic fibers which are thicker than those of the endocardium proper and generally run at right angles to the latter. The outermost zone of this subendocardial layer may at times take on a looser structure and merge with the collagenous framework of the adjacent auricular myocardium. A few scattered capillaries and very rare mononuclear cells are normally present in the subendocardium. Lymphocytes and polymorphonuclear leukocytes are practically never seen. The capillaries are often more conspicuous when fat tissue is present.

During the second year of life the regularity of the endocardial pattern may be somewhat disturbed. The elastic fibers may show focal concentrations or, in places, be missing. Thus, the beginnings of a mosaic pattern are already noted. This becomes more frequent during the second half of the first decade. About the end of the first decade an occasional specimen shows a somewhat greater concentration of smooth muscle fibers in the outermost endocardial zone. These run occasionally obliquely, but for the most part, transversely. At times the innermost endocardial layers may be scant in elastic fibers and present an appearance somewhat resembling the inflammatory structure which will be referred to as an "endocardial reduplication." These are, however, generally easily distinguishable from true reduplications during the first four decades of life, after which they occur with considerably greater frequency and can seldom be distinguished from the type of reduplication often found in chronic valvular disease of long duration.

From the second decade on, occasional specimens show a less distinct differentiation between the endocardium and subendocardium. During the third decade the subendocardium begins to show fat accumulations. As indicated before, these frequently contain a larger number of capillaries. The smooth muscle fibers, which gen-

erally occupy the outer zone of the endocardium proper and occur in more or less compact masses, become decidedly more conspicuous in the fourth decade and may produce irregularities in the distribution of the elastic lamellae, with the formation of elastic-muscular mosaics.

In the sixth decade of life a new pattern appears. The superficial layers of the elastic tissue may be either concentrated or quite defective. The inner zone of the endocardium generally shows atrophy of the elastic fibers with, at times, disappearance. The middle zone often shows dense concentrations of elastic fibers. The outer zone frequently contains conspicuous smooth muscle bundles, at times intermingled with dense elastic concentrations. The subendocardium frequently contains considerable fat tissue.

In the seventh decade the elastic fibers become extremely scant, being concentrated in irregular collections; the smooth muscle components are quite conspicuous and endocardial reduplications internal to the innermost elastic lamellae are frequently encountered. These reduplications are generally narrow, not elastified, of more or less even thickness and rest on a fairly heavy, elastic limiting membrane. The eighth decade frequently shows distinct atrophy of the endocardium with, generally, hypertrophy of the smooth muscle elements.

MACROSCOPIC APPEARANCE OF RHEUMATIC AURICULAR ENDOCARDITIS

The macroscopic appearance of the rheumatic auricular lesions has been described in detail by VonGlahn.¹⁵ The chief points brought out by this author are the predilection site of the posterior wall of the left auricle, at times spreading over almost the entire endocardial surface into the auricular appendage and up to the orifices of the pulmonary veins; the rare involvement of the foramen ovale; the appearance of irregular low ridges and hillocks, separated by furrows with no definite pattern; the smooth, glistening, at times slightly dull, irregular surface with, rarely, distinct projections resembling vegetations; the tawny gray color in the more acute lesions and grayer and translucent appearance of the older ones; and in the more extensive process, the flat or delicately ridged plaques of yellow color.

To the above résumé may be added the fact that not infrequently the only macroscopic evidences of endocardial lesions are the presence of very inconspicuous, flat, often rounded plateaus, sometimes measuring no more than 2 mm. in diameter, generally delicate pink but, at times, no different in color from the remainder of the left auricular endocardium. These delicate elevations merge imperceptibly with the adjacent tissues and are best brought out by sponging the inner surface of the auricle and allowing the light to fall on the lesion in such a way as to emphasize the shadows, much as is done in the photography of flat projections.

It is to be noted that the normal endocardium often displays a cross weaving of elastic fibers in somewhat sharply cut geometric pattern. These may appear as slightly raised, white or gray ridges which, however, are practically always in the form of straight lines. The flat rheumatic auricular lesions differ from these geometric elastic patterns of the normal auricular endocardium in their rounded contour, greater irregularity, broader base and, sometimes, in their pinkish color.

The only statistics available on the incidence of macroscopic left auricular rheumatic lesions are those by VonGlahn, who observed 9 instances among 31 cases (29 per cent), and Thayer,²⁸ who noted acute or chronic mural endocarditis of the left auricle in 10 out of 25 cases (40 per cent). Gross auricular lesions were observed in 80 per cent of the series of rheumatic hearts which form the basis of this report. The highest incidence was in Group 3 of the above mentioned clinical classification (repeated attacks) where they were found in every instance. The lowest incidence was in Group 5 (chronic valvular disease without Aschoff bodies) where they were found in approximately half the cases. Macroscopic lesions in the right auricle were very mild, not nearly as easily discernible as those of the left auricle and relatively infrequent. Their histological characteristics are similar to those of the left auricular lesions.

MICROSCOPIC APPEARANCE OF RHEUMATIC AURICULAR LESIONS

As has been indicated by previous authors, the auricular lesions in rheumatic fever may present a variety of pictures depending on the acuity of the process and on the individual reactions of the tissues. These reactions in turn may possibly be influenced by the immuno-

logical state of the individual. An analysis of these histological changes indicates that they may be roughly classified into five types, each corresponding to one of the clinical groups mentioned above.

Group 1

Histology of the Left Auricular Lesion Found in Active Cases Where the Individual Died Either in the First Attack or Where One Preceding Attack Occurred Within 1 Year of the Fatal Outcome

There were 17 cases in this group, ranging in age from 17 months to 19 years. The endocardium proper in every specimen showed varying grades of infiltration with inflammatory cells (Fig. 2). These were generally polymorphonuclear leukocytes and lymphocytes, as well as occasional eosinophiles, plasma cells and large mononuclear cells. Many of the cells were oriented at right angles to the auricular lumen, appearing often as ameboid streamers. In approximately half the cases the infiltration was very marked. In almost every specimen there were noted palisades or banded arrangements of these cells on either side of swollen elastic and collagen fibers. There did not appear to be any site of predilection within the auricular endocardium for these palisades. Apart from these, however, the infiltration was generally diffuse with numerous, focal, intense accumulations scattered irregularly throughout the several zones of the endocardium. In approximately half the cases there were present stratified cellular accumulations with histological properties similar to the cellular components entering into the formation of Aschoff bodies. Thus, the cells possessed owl-eyed, fibrocytoid and pyknotic nuclei and were surrounded by rather abundant basophilic cytoplasm with ragged edges. These cells also appeared to be limited by the adjacent elastic and collagenous bands (Fig. 3) and sometimes arranged themselves around fragments or sheets of swollen eosinophilic collagen. These lesions will be referred to as endocardial Aschoff bodies.

Besides these cellular accumulations the endocardium, as indicated, generally showed conspicuous bands and foci of swollen eosinophilic collagen (fibrinoid change). The elastic fibers almost invariably showed a definite departure from the normal consistence and topography. Instead of the regular lamellated arrangement seen in normal controls, particularly in the younger age periods,

many of the endocardial lesions showed spreading apart of the elastic lamellae (Fig. 4) and deviation from their parallel arrangement so that arches were formed, often directed toward the subendocardium. Occasionally, the membranes showed rupture with irregular patches of thinning; in other areas they were thickened and concentrated (Fig. 5). For simplicity of description such irregularities in the distribution of the elastic membranes will be referred to as "elastica distortions." Approximately one-third of the cases showed marked edema of the endocardium. This generally occurred in the area showing greatest cellular accumulations.

As previously indicated, there is a tendency for the smooth muscle of the endocardium to increase with advancing age. This muscular stratum is generally but not invariably situated in the outermost zone of the endocardium and usually occurs in more or less compact form. In the typical auricular lesion falling in this clinical category, the smooth muscle elements may increase in number (Figs. 6 and 7). They are sometimes irregularly arranged; the bundles may be separated into smaller discrete islands and occupy various zones of the endocardium proper.

It has also been shown that the normal endocardium possesses no discernible capillaries (as opposed to the subendocardium, in which capillaries are invariably seen). In the auricular lesion under description there is seen in the majority of instances a penetration of capillaries from the subendocardium into the endocardium proper. These are frequently seen to lie between the smooth muscle bundles, often arranged at right angles to the endocardial surface (Fig. 7). In approximately half the cases they penetrate to the middle zone of the endocardium (Figs. 8 and 10) and in about one-third of the cases they penetrate as far as the inner zone (Fig. 9). This phenomenon will be referred to as "capillary penetration," although it is to be noted that in a few instances the penetrating vessels are of arteriolar structure.

Perhaps the most important feature of this lesion is the endocardial "reduplication." This is a term intended to designate the formation of a new layer of tissue situated between the innermost elastic lamella and the auricular endothelial lining. These reduplications occur in several different forms. In the clinical group under discussion the most frequent form is the embryonal uncovered type. This consists of a not inconspicuous layer of mucinoid-staining mate-

rial which may attain a width approximately equal to that of the endocardium proper (Fig. 6). Within the matrix of this material there are seen stellate and spindle cells often running at right angles to the elastic membranes and consisting of vesicular nuclei surrounded by a rather hazy, faintly staining cytoplasm. Inasmuch as these cells apparently possess the multipotentiality of mesenchymal tissue (transforming themselves usually into fibroblasts, smooth muscle and, rarely, bone) they are referred to here as embryonal mesenchymal cells. Occasionally in this group an elastic membrane is seen covering this reduplication (covered embryonal reduplication). It is apparently this lesion that VonGlahn considered one of the healing processes of the endocardial lesion.

In this clinical group more than half the cases show these reduplications as single strata. Occasionally, however, two strata are seen separated from one another by an elastic membrane. These are referred to as multiple reduplications (Figs. 6, 10 and 12). Another form of reduplication is the edematous type. This was seen only once and consisted of a rather gelatinous tissue infiltrated with small, round, inflammatory cells (Fig. 4). Although occurring infrequently in this group, the covered reduplication presents another variant. This consists of an endocardial reduplication, often with an embryonal matrix, lying inside the innermost auricular endocardial elastic membrane and showing various grades of elastification with more or less parallel rows of elastic lamellae (elastified reduplication) (Fig. 8). Another form of reduplication is the dense collagenous variety. As its name indicates, this consists of rather dense collagen bundles (Figs. 9, 10 and 11) which may or may not be penetrated with a varying amount of elastic tissue. This occurred only once in this series. Smooth muscle cells in the endocardial reduplications were seen only once in this group (Fig. 10).

In this clinical group the subendocardium invariably shows evidence of inflammatory change (Figs. 2, 3 and 4). This generally consists of a marked infiltration between the collagenous bundles. The latter may or may not show eosinophilic swelling. The cellular components are similar to those mentioned as forming the characteristic infiltration of the endocardium. The infiltration is generally diffuse but may be focal. Both the inflammatory cells as well as edema produce, at times, a marked increase in the width of the subendocardium (Figs. 3 and 4). In approximately one-third of the cases Aschoff

bodies were present in the subendocardium, these being of a somewhat modified mosaic pattern with the characteristic cells lying in the crypts between the collagenous bundles. The elastic fibers of the subendocardium also show considerable modification, such as thickening, swelling, rupture, concentration and, at times, disappearance.

The capillary component of the subendocardium generally shows a very definite increase in their number. Not infrequently they are larger, more conspicuous, often oriented toward the auricular lumen, sometimes with endothelial buds and swollen endothelial cells. One case in this group showed the characteristic, intimal, musculo-elastic hyperplastic changes similar to those described by Gross, Kugel and Epstein²⁹ as occurring in other coronary vessels in the heart in rheumatic fever (Fig. 11). Not infrequently delicate vascular channels are seen to be distended with lymphocytes (Fig. 1). This was pointed out by MacCallum.

Approximately half the cases showed considerable hypertrophy of the myocardium (Fig. 12). As Sacks³⁰ has indicated, such hypertrophy is not necessarily associated with valvular defects but appears to be a direct stimulating effect of the exciting agent. In the interstitium between the myocardial bundles several cases showed edema and early fibrosis. Practically every specimen showed some form of cellular infiltration, often with lymphocytes, sometimes with polymorphonuclear leukocytes, occasionally plasma cells, eosinophiles and large mononuclear cells (Fig. 1). These were arranged either diffusely or focally and very often showed a contiguity with the subendocardial infiltration on the one hand and with an inflammation of the pericardium on the other. Apart from these non-specific cellular infiltrations, more than one-third of the specimens showed myocardial Aschoff bodies of various types.

As indicated in a previous report,²⁹ the vascular lesions occurring in the left auricular wall in acute rheumatic fever are not conspicuous or characteristic. Nevertheless, the majority of cases showed vessels with glassy medial hypertrophy, and with medial hypertrophy.* In some cases the capillaries were rather conspicuous. Rarely giant medial hypertrophy with metallaxis was encountered. In 2 cases intimal fibrosis was noted.

In every specimen some form of pericardial inflammation was

* For a description of these lesions see Gross, Kugel and Epstein.²⁹

noted. In more than half the cases this consisted of a microscopic, mild or marked infiltration, generally with lymphocytes, occasionally with polymorphonuclear leukocytes, eosinophiles, plasma cells and large mononuclear cells. These infiltrations tended to occupy the outermost zones of the pericardium. In 2 cases pericardial Aschoff bodies were seen. Rarely, eosinophilic swelling of the collagen was noted. The pericardial capillaries were generally quite conspicuous. In 2 cases the vessels showed small thrombi, in 1 case there was seen polypoid endarteritis, giant medial hypertrophy with metallaxis and intimal musculo-elastic hypertrophy.

In one-third of the cases a distinct fibrinous pericarditis was present. It is to be noted, however, that this figure represents the incidence of this lesion as it was observed in the left auricular section studied. Macroscopic pericarditis, either fibrinous or adhesive, was observed on some portion of the entire heart in 80 per cent of the cases in this clinical group.

Group 2

Histology of the Left Auricular Lesion Found in Active Cases Where One Previous Attack Occurred at Least 2 Years Previous to the Fatal Outcome

There were 10 cases in this group, ranging in age from 7 to 34 years. The average endocardial lesion resembled very closely that described for the first group. For the sake of simplicity its chief histological features are listed seriatim as follows:

1. Palisades were observed in only 4 of the 10 cases, as compared to 15 of the 17 cases in Group 1. These palisades did not differ essentially in their histological characteristics from those previously described. The same may be said for the incidence of eosinophilic swelling of the collagenous tissue, which closely paralleled the incidence and extent of the cellular infiltration.
2. Endocardial Aschoff bodies were found of the same type as those previously described. The incidence, however, was appreciably lower (2 out of 10 cases).
3. Inflammatory cell infiltrations, elastica lesions, capillary penetration and increase in the smooth muscle component of the endocardium were similar to that described in Group 1.

4. The most conspicuous difference from the first group lies in the nature and incidence of the reduplications. These were found in 7 out of the 10 cases. Multiple reduplications were seen in 1 case. All the reduplications were elastified. Some were covered. Two were of the dense collagenous variety and one showed a smooth muscle component.
5. As in Group 1, the width of the subendocardium was increased in approximately half the cases (Fig. 9).
6. Infiltration of the subendocardium with inflammatory cells occurred with about the same frequency as in Group 1, with perhaps a lower grade of intensity in approximately half the cases.
7. Subendocardial Aschoff bodies were found in only 1 case of the 10, as compared to 5 of the 17 in Group 1.
8. The increase in subendocardial vascularization was approximately the same as in Group 1, as was the nature of the vessels. One case showed arterioles and arteries with hypertrophied walls.
9. Eight out of the 10 cases showed myocardial hypertrophy. In the majority of these the hypertrophy was marked.
10. The interstitial myocardial infiltration, which was on the whole somewhat less marked than that in Group 1, occurred in 7 out of the 10 cases. Aschoff bodies were found in 4 cases.
11. Myocardial fibrosis was of about the same extent and incidence as in Group 1.
12. The myocardial vascular lesions were somewhat more varied. Thus, in 2 cases some of the blood vessels showed proliferation and desquamation of the endothelium; 1 case showed giant medial hypertrophy with metallaxis; 2, intimal fibrosis; 2, plugging of vessels with small thrombi; 1, intimal musculo-elastic hyperplastic changes, and 1, intimal elastification even in an early age period.
13. The incidence and type of microscopic pericarditis is similar to that described in Group 1. In 2 cases section of the left auricle showed adhesive pericarditis; 1 case showed a variety of rheumatic vascular lesions in the pericardium similar to those described in Group 1. (Macroscopic pericarditis, either fibrinous or adhesive, was observed on some portion of the entire heart in 7 of the 10 cases in this clinical group.)

Taken as a whole, the most conspicuous differences between the left auricular lesions in the first group and those in this clinical group lie in the lower incidence in the latter of palisade formations and of Aschoff bodies in the endocardium, subendocardium and myocardium; in the somewhat higher incidence and qualitative differences in the reduplications; and in the milder form of the interstitial myocarditis.

Group 3

Histology of the Left Auricular Lesion Found in Active Cases With Repeated Attacks, Death Occurring During an Acute Recurrence

There were 12 cases in this clinical group, ranging in age from 4½ to 36 years. The average endocardial lesion showed decidedly greater changes from the type described in Group 1 than did the previously described group. The following are the chief histological features:

1. While infiltration of the endocardium, as in the previous groups, was found in every case, this was marked in only 5 cases. The character of the cells and the incidence of eosinophilic swelling of the collagen was about the same. As in the previously described group, palisades were found less frequently than in Group 1. These were noted in 7 cases, in 5 of which the cells were of the large mononuclear variety.
2. Endocardial Aschoff bodies were found in 2 cases.
3. The elastica lesions were similar to those described in Group 1. They were found in every case.
4. Capillary penetration occurred in 11 of the cases. In 4 this was quite marked. In the rest of the cases penetration was very inconspicuous and affected only the outermost musculo-elastic zone.
5. The smooth muscle increase was perhaps more definite in this group.
6. The most important difference from the previous groups lay in the incidence and type of reduplications. Thus, 6 cases of the 12 showed multiple reduplications, of which 3 possessed triple reduplications. Altogether, reduplications were found in 11 cases. The majority of them were elastified. A few of them were of the embryonal type and 2 showed smooth muscle components.

7. In practically every instance the subendocardium was somewhat increased in width. The infiltration, which was qualitatively of the same type as previously described, was marked in 7 cases.
8. In 3 cases subendocardial Aschoff bodies were present.
9. The increase in subendocardial vascularization, though still high in incidence (9 cases), was somewhat lower than previously described.
10. Eleven of the 12 cases showed marked myocardial hypertrophy.
11. Fibrosis between the myocardial bundles was found in 7 of the 12 cases, in 1 of which elastification was also present, even though in a relatively early age period (17 years).
12. In 4 cases inflammatory cell infiltration was marked. In 3 there was no infiltration noted. In the rest the infiltration was mild. Myocardial Aschoff bodies were found in 2 cases.
13. Vascular lesions of the myocardium occurred in even greater variety and frequency in this group. Thus, 3 cases showed intimal elastification; 2, intimal fibrosis; 4, thickening of the myocardial arteries or arterioles; and 1, intimal musculo-elastic hyperplastic changes.
14. Eight cases showed organized or organizing pericarditis, a much higher incidence than was found in the previous groups. Typical rheumatic vascular lesions were found in 1 case. (Macroscopic pericarditis, either fibrinous or adhesive, was observed on some portion of the entire heart in 10 of the 12 cases in this clinical group.)

Considered as a whole, the features of this group are the tendency for the palisade formations to consist of large mononuclear cells, the higher incidence of multiple reduplications and adhesive pericarditis, and the lower incidence of interstitial myocarditis.

Group 4

Histology of the Left Auricular Lesion Found in Active Cases Where Death was Caused by Decompensation Without Clinical Evidence of a Final Recurrence. Some of these Cases Had No Previous History of Rheumatic Fever

There were 28 cases in this clinical group, ranging in age from 18 to 62 years. The average auricular lesion showed a decided decrease in active inflammatory phenomena. The following are the chief histological features:

1. Only 5 cases of the 28 in this group showed marked infiltrations of the endocardium; these were qualitatively similar to those described previously. Five cases showed no infiltration. In the remaining cases there was present only a mild grade of generally lymphocytic infiltration. Edema was also less marked and less frequent in this group.
2. Endocardial Aschoff bodies were found in only 4 of the 28 cases.
3. The incidence of palisade formations was decidedly lower, only 3 cases presenting these lesions. These were of the larger mononuclear cell variety. Eosinophilic swelling of the collagen occurred in only 3 cases, in 1 of which it was quite pronounced.
4. The smooth muscle components of the endocardium were very definitely increased, even after making allowance for the fact that the majority of the cases in this group belong to older age periods.
5. The elastica lesions were of the patchy variety and somewhat difficult to distinguish from age period changes (Fig. 11).
6. Nine cases showed multiple reduplications, of which 3 were triple reduplications. Altogether, 27 cases showed reduplications, generally of the elastified or dense collagenous variety. These were usually covered. In 1 case (Fig. 11), the reduplication was of the flat, collagenous, and somewhat elastified variety which, as will be noted later, is very characteristic of the cases belonging to the next clinical group, *i.e.* chronic valvular disease without activity. One

reduplication was of the embryonal type. Three of the reduplications showed smooth muscle components. One case showed calcific deposits on the superficial layers of the endocardium.

7. The width of the subendocardium was increased in the majority of instances.
8. Five of the 28 cases showed marked cellular infiltrations of the subendocardium; 17 cases showed mild infiltrations; 6 cases showed none.
9. Subendocardial Aschoff bodies were found in 7 of the 28 cases.
10. The vascularization of the subendocardium was increased in the majority of the cases. In 1 there was noted arteriolar hypertrophy; intimal musculo-elastic hyperplastic lesions were noted in 2.
11. In practically every case the myocardium was markedly hypertrophied.
12. In 18 cases there was no infiltration of the interstitial tissue of the myocardium. When it occurred it was generally mild and focal, consisting usually of lymphocytes and histiocytes. In 1 case myocardial Aschoff bodies were present.
13. The incidence of myocardial fibrosis was similar to that previously described. In addition, however, elastification occurred in 3 cases (in the somewhat later age periods).
14. The vascular lesions of the myocardium consisted of intimal elastification in about half of the cases. It is to be noted again that these occurred in the somewhat later age periods.
15. The pericardial lesions were on the whole mild. However, fibrinous pericarditis occurred in 1 case. Peculiar infoldings of the pericardial mantle with large swollen lining cells were found in 2 cases. Pericardial Aschoff bodies were found in 1 case. (Macroscopic pericarditis, either fibrinous or adhesive, was observed on some portion of the entire heart in 9 out of the 28 cases in this clinical group.)

Considered as a whole, the features of this group are the lower incidence of endocardial infiltration, palisade formation, Aschoff bodies and myocardial infiltration, and the higher incidence and peculiarity of the reduplications as well as the mildness of the pericardial lesions.

Group 5

Histology of the Left Auricular Lesion Found in Inactive Cases of Chronic Valvular Disease of the Typical Rheumatic Variety

There were 20 cases in this clinical group, ranging in age from 11 to 80 years. The average auricular lesion was marked by its extreme indolence. The following are the chief histological features:

1. Infiltrations of the endocardium occurred in practically every specimen. However, these were all very mild, consisting of scattered lymphocytes, occasional amebocytes and often large mononuclear cells. In many instances these infiltrations could not be distinguished from the occasional cellular infiltrations of the normal endocardium. However, inasmuch as the latter showed practically no infiltration after the fourth decade, its occurrence in many of the older cases of this clinical group was of some significance.
2. In 1 case there occurred a palisade formation of large mononuclear cells. This was associated with a moderate amount of eosinophilic swelling of the collagen. Edema was not found.
3. Aschoff bodies were not present in the endocardium.
4. A great variety of elastic changes was found in this group. None of them, however, showed the characteristic separation and stretching of the elastic fibers seen in the more active lesions. They consisted generally of exaggerations of the normally occurring age period changes of the elastic tissue, from which they were difficult and often impossible to distinguish. On the whole, it may be said that the elastic distribution in the endocardium in cases falling into this group is distinctly irregular, patchy, with, not infrequently, areas of cross-weaving of the elastic fibers and accumulation into compact bundles forming a mosaic with areas in which the elastic tissue was extremely sparse.
5. As in the previously described groups, the smooth muscle of the endocardium was increased. This, however, was frequently difficult to judge because of the older age periods in which most of these cases fell.
6. The endocardium itself was lightly infiltrated with calcium salts in 1 case.

7. All the cases showed reduplications. In 2 cases these were multiple. In the great majority of cases the reduplications were of the flat, dense, elastified variety. Smooth muscle was found in one reduplication.
8. Many of the specimens showed a moderate widening of the subendocardium. This consisted frequently of fibrosis, to which there was often added a fat cell component.
9. Infiltration of the subendocardium was generally very mild and consisted of lymphocytes. In the somewhat younger cases the infiltration tended to be slightly more conspicuous. Two cases showed no infiltration. No subendocardial Aschoff bodies were present.
10. About half the cases showed increase in vascularization without distinctive features.
11. All the cases showed a very marked hypertrophy of the auricular myocardium.
12. Infiltration of the myocardial interstitium was even less marked than in the previous group. It occurred in approximately half the cases and was generally mild and focal.
13. Fibrosis of the myocardium occurred in about one-third of the cases.
14. The most conspicuous myocardial vascular lesion was congestion of the capillaries, which occurred in about one-third of the cases. For the rest, the arterioles appeared to be somewhat hypertrophied and intimal elastification was somewhat more frequently found. However, these corresponded to the age period changes to be expected in this group.
15. Pericardial lesions were found in only 12 cases and consisted of mild scattering of lymphocytes. (Macroscopic pericarditis, either fibrinous or adhesive, was observed on some portion of the entire heart in 4 out of the 20 cases in this clinical group.)

Considered as a whole, this group of cases is notable for the extreme mildness of infiltrative phenomena, for the absence of Aschoff bodies and for the possession of the characteristic, flat, elastified reduplications.

DISCUSSION

Although the blocks of tissue on which this study was made represent single specimens from each case cut according to our routine procedure and generally without any special effort to include macroscopic lesions, every specimen showed some variety of histological lesion that can be reasonably attributed to rheumatic fever. It must not be inferred from this, however, that the lesions were always so distinctive as to permit a diagnosis of rheumatic fever solely on the auricular findings. Particularly in Groups 4 and 5 it is not infrequently difficult to distinguish the essential inflammatory lesions of rheumatic origin from the confusing concomitant age period changes. On the other hand, in the clinical groups which represented the more active cases (Groups 1, 2 and 3) the lesions were very varied, generally quite conspicuous and presented a sufficient number of individual pathological processes on which it is possible to make a diagnosis of rheumatic fever. These lesions consist of edema and marked infiltrations of the endocardium with inflammatory cells, the banded appearance of some of these cellular aggregations, the presence of eosinophilic swelling of the collagen, the presence of Aschoff bodies in the endocardium, subendocardium and myocardium, the distortion of the elastic tissue and the widening, hypercapillarization and marked infiltration of the subendocardium. To these, furthermore, may be added the two important features of capillary penetration into the endocardium and the presence of reduplications. A less important feature is the presence of scattered and increased smooth muscle elements in the endocardium, together with smooth muscle in the reduplications proper. In none of the material examined was there present a necrosis of the superficial layers of the endocardium of sufficient intensity to warrant the term "verrucous change." Together with these endocardial and subendocardial lesions these first three groups also presented a very high incidence of myocardial hypertrophy and interstitial inflammatory cell infiltration. In approximately half the cases microscopic section of the left auricle showed some pericardial lesion.

In the more chronic clinical groups (4 and 5) the inflammatory phenomena of the endocardium and subendocardium were present in almost every instance, but very much milder. Aschoff bodies occurred in extremely low incidence or not at all (Group 5), and the

subendocardial inflammatory phenomena were also less frequent and milder, as were those in the myocardium and pericardium. On the other hand, the almost invariable presence of reduplications, sometimes multiple, the penetration of capillaries into the endocardium, when present, the marked hypertrophy of the myocardium and the presence in the majority of cases of some form of pericardial lesion, together with the increase and irregularity of the endocardial smooth muscle, readily constituted criteria on which to entertain at least a suspicion of rheumatic fever.

Of great interest is a consideration of the differences in the microscopic lesions between each of the five clinical groups. Certain phenomena are observed throughout all the groups, even though usually in different proportions. On the other hand, each group presents certain characteristics of its own which are both of a quantitative as well as of a qualitative value. Thus, active inflammation of the endocardium and subendocardium, as well as of the myocardium, is generally noted in Groups 1, 2 and 3. These inflammations are less marked in Group 4 and extremely mild in Group 5. This is true for the cellular infiltrations, edema and eosinophilic collagen swelling. The incidence of small round cell inflammatory infiltration shows a rather abrupt decline in Groups 4 and 5 where large mononuclear cells are more frequently found. As previously noted, palisades were observed with considerable frequency in Groups 1, 2 and 3. They were infrequent in Group 4 and occurred only once in Group 5. The same may be said of the incidence of Aschoff bodies which was high in only the first group. Capillary penetration of the endocardium is seen in all the groups, being most marked, however, in the first four. As noted, inflammatory infiltrations of the subendocardium occur in all the groups, but are most marked in the first three. Increased vascularization of the subendocardium is found in all the groups, chiefly in Groups 2, 3 and 4. The incidence and type of pericarditis varies somewhat. It is somewhat similar in the first two groups, where it is generally of the lymphocytic or fibrinous variety. Organizing pericarditis, however, appears conspicuously in Group 3. The total incidence falls in Group 4 and especially in Group 5.

Besides the conspicuous difference in the incidence of Aschoff bodies, cellular infiltration, palisade formation and eosinophilic swelling of collagen, the incidence and types of endocardial redupli-

cations constitute the most characteristic difference between each of the five groups. Thus, in Group 1, 10 of the 17 cases showed reduplications usually single and of the embryonal, uncovered variety. In Group 2 the incidence is higher (7 out of 10), the reduplications are generally single but the type is changed to either the elastified or covered variety. In Group 3 multiple reduplications begin to make their appearance. The majority of lesions are elastified. Some are triple. In Group 4 the reduplications are multiple in approximately one-third of the cases; in the majority they are elastified or dense and covered. In Group 5 the great majority of the reduplications are of the flat, dense, elastified variety. It is seen, therefore, that the appearance of these reduplications constitutes one of the most significant differences in the lesions peculiar to the several clinical groups of rheumatic fever.

In spite of the different histological features of the several clinical groups of rheumatic fever pointed out in this report, it must be borne in mind that the observations were made on too limited a number of cases on which to place complete reliance on the statistics submitted, nor indeed is it considered justifiable as yet to attempt such fine distinctions in the appraisal of a given case. The observations are presented as a suggestive indication for further study and with the thought that other associated lesions in the heart and elsewhere in the body as a whole may reflect, at least to a certain extent, the differences in the reactions of the tissues to rheumatic fever as determined by the clinical course of the disease.

SUMMARY AND CONCLUSIONS

Gross and histological observations on the left auricle, based on an examination of 87 rheumatic hearts, are described. The material is classified into five clinical groups, depending on the course of the disease. It is shown that macroscopic lesions of the left auricle occur in 80 per cent of the cases and microscopic lesions in 100 per cent. In the acute cases the lesions are very significant and characteristic. In the chronic cases they are considerably milder and often difficult to differentiate from normally occurring histological changes. A description is also given of the age period changes of the normal left auricle, as observed in 50 hearts.

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DESCRIPTION OF PLATES

PLATE 100

FIG. 1. Normal left auricle. Age 3 months. Low power. Weigert's elastic and Van Gieson's connective tissue stain.

Arrow indicates division between A, endocardium and B, subendocardium; C = myocardium.

FIG. 2. Left auricle from active case of rheumatic fever. Age 18 years. Low power. Hematoxylin and eosin stain.

A = edematous portion of endocardium; B = infiltration of endocardium with inflammatory cells in banded arrangement; C = markedly infiltrated subendocardium. Note hypercapillarization. D = marked infiltration of myocardial interstitium.

FIG. 3. Left auricle from active case of rheumatic fever (injected specimen). Age 12 years. Low power. Hematoxylin and eosin stain.

A = endocardium infiltrated with small round cells. Note palisade formations. B = palisade formation of the endocardial Aschoff body type. C = considerably widened subendocardium. Note infiltration with inflammatory cells and hypercapillarization with many capillaries oriented at right angles to endocardial surface. D = myocardium with distended capillaries, and shows mild interstitial infiltration.

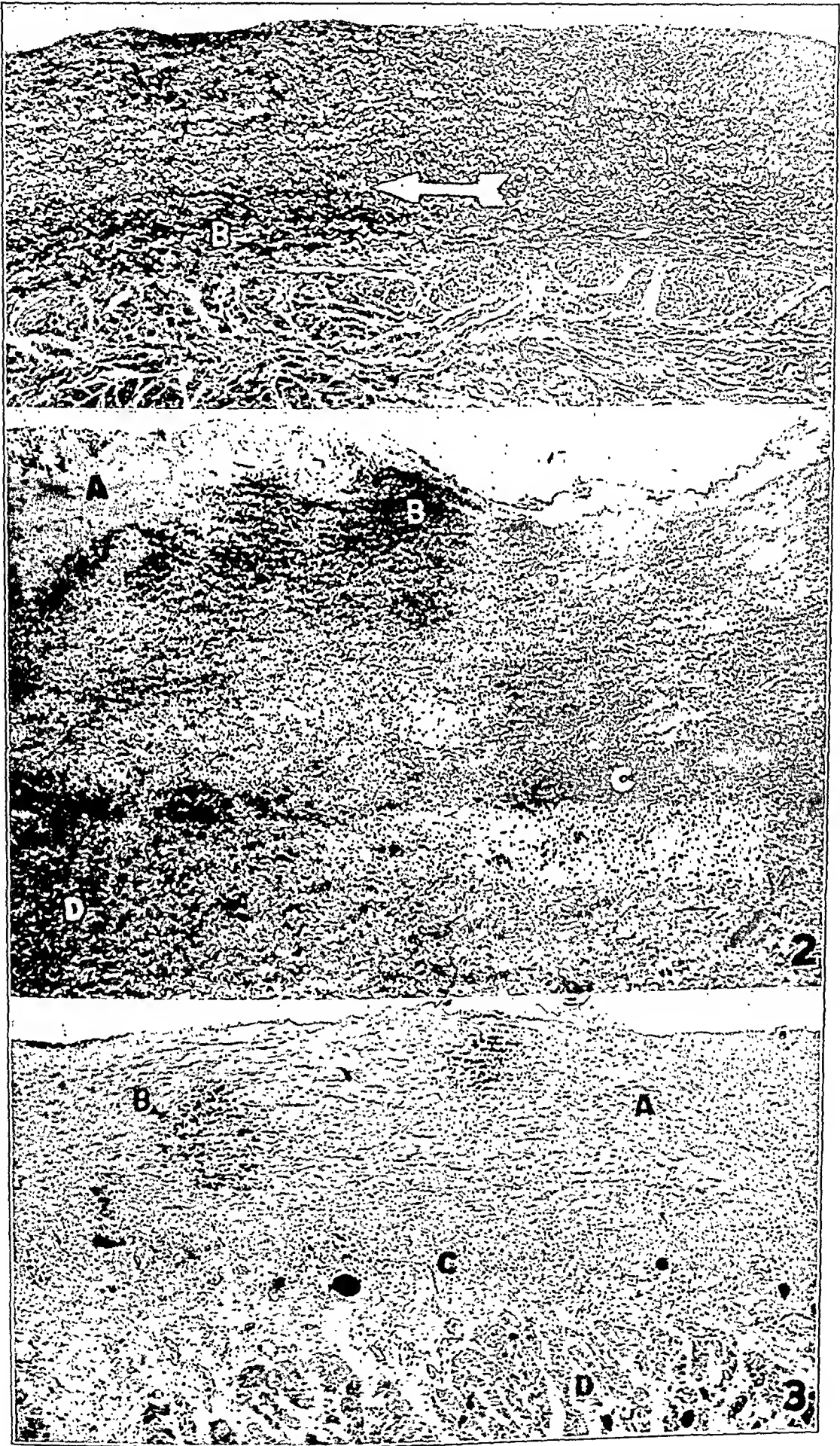


PLATE 101

FIG. 4. Left auricle from active case of rheumatic fever. Age 18 years. Low power. Weigert's elastic and Van Gieson's connective tissue stain.

A = endocardial reduplication of edematous type. Note the small round cell inflammatory infiltration of this reduplication and the condensation of the elastic lamellae beneath it. B = endocardium markedly infiltrated with small round cells. Note edema, stretching and separation of elastic fibers. C = markedly inflamed, edematous and widened subendocardium.

FIG. 5. Left auricle from active case of rheumatic fever. Age 12 years. Low power. Weigert's elastic and Van Gieson's connective tissue stain.

A = endocardium showing distortion and condensation of elastic fibers; B = condensation of elastic fibers in subendocardium; C = edematous infiltrated subendocardium with hypercapillarization.

FIG. 6. Left auricle from active case of rheumatic fever showing multiple reduplications. Age 13 years. Low power. Weigert's elastic and Van Gieson's connective tissue stain.

A = older reduplication of embryonal covered type; B = more recent reduplication of partially covered embryonal type; C = uncovered portion of reduplication; D = endocardium proper showing marked edema, paucity of elastic fibers and mild small round cell infiltration; E = considerable increase in smooth muscle component of outer third of endocardium. Note irregularity of smooth muscle bundles, their separation by connective tissue and F, capillary penetration toward middle third of endocardium. G = markedly widened subendocardium with mild small round cell infiltration and marked hypercapillarization; H = myocardium.

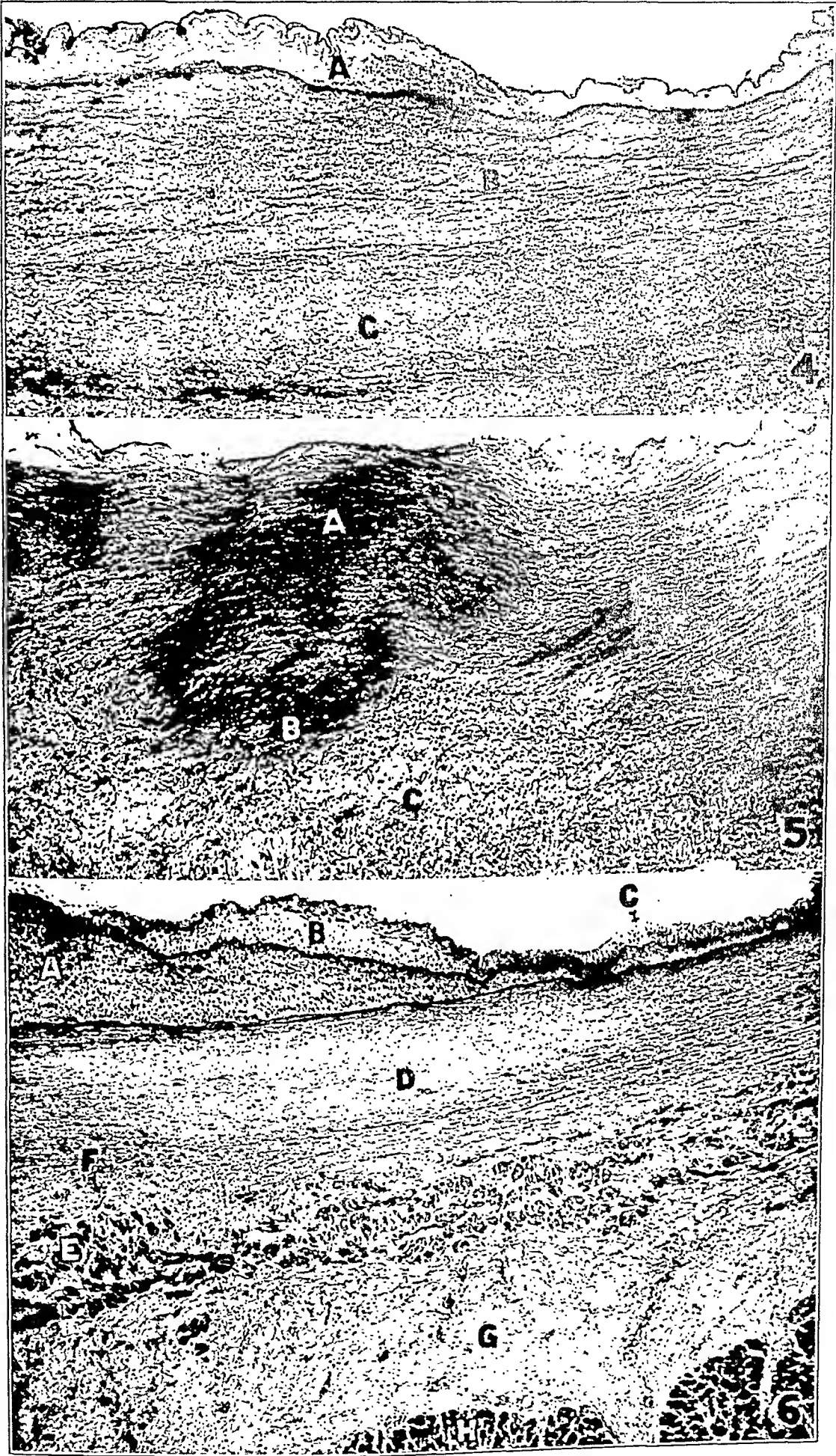


PLATE 102

FIG. 7. Smooth muscle zone in left auricle from active case of rheumatic fever. Age 13 years. High power. Masson's erythrosine-saffron stain. Note considerable increase in smooth muscle cells with irregularity in arrangement.

A = smooth muscle bundle; B = large amount of collagenous tissue between dispersed smooth muscle bundles; C = penetrating capillaries oriented at right angles to endocardium.

FIG. 8. Left auricle from active case of rheumatic fever. Age 15 years. Low power. Weigert's elastic and Van Gieson's connective tissue stain.

A = elastified reduplication with suggestion of origin from multiple reduplications; B = inflamed endocardium with capillary penetration to middle third; C = considerably widened, inflamed and hypercapillarized subendocardium; D = myocardium.

FIG. 9. Left auricle from active case of rheumatic fever (injected specimen). Age 7 years. Low power. Weigert's elastic and Van Gieson's connective tissue stain.

A = irregular collagenous reduplication with large vascular channels and some small round cell infiltration; B = capillary penetration of endocardium as far as the limiting membrane overlying the inner third; C = middle zone of endocardium showing marked elastica distortion and capillary penetration; D = widened subendocardium with hypercapillarization; E = myocardium.

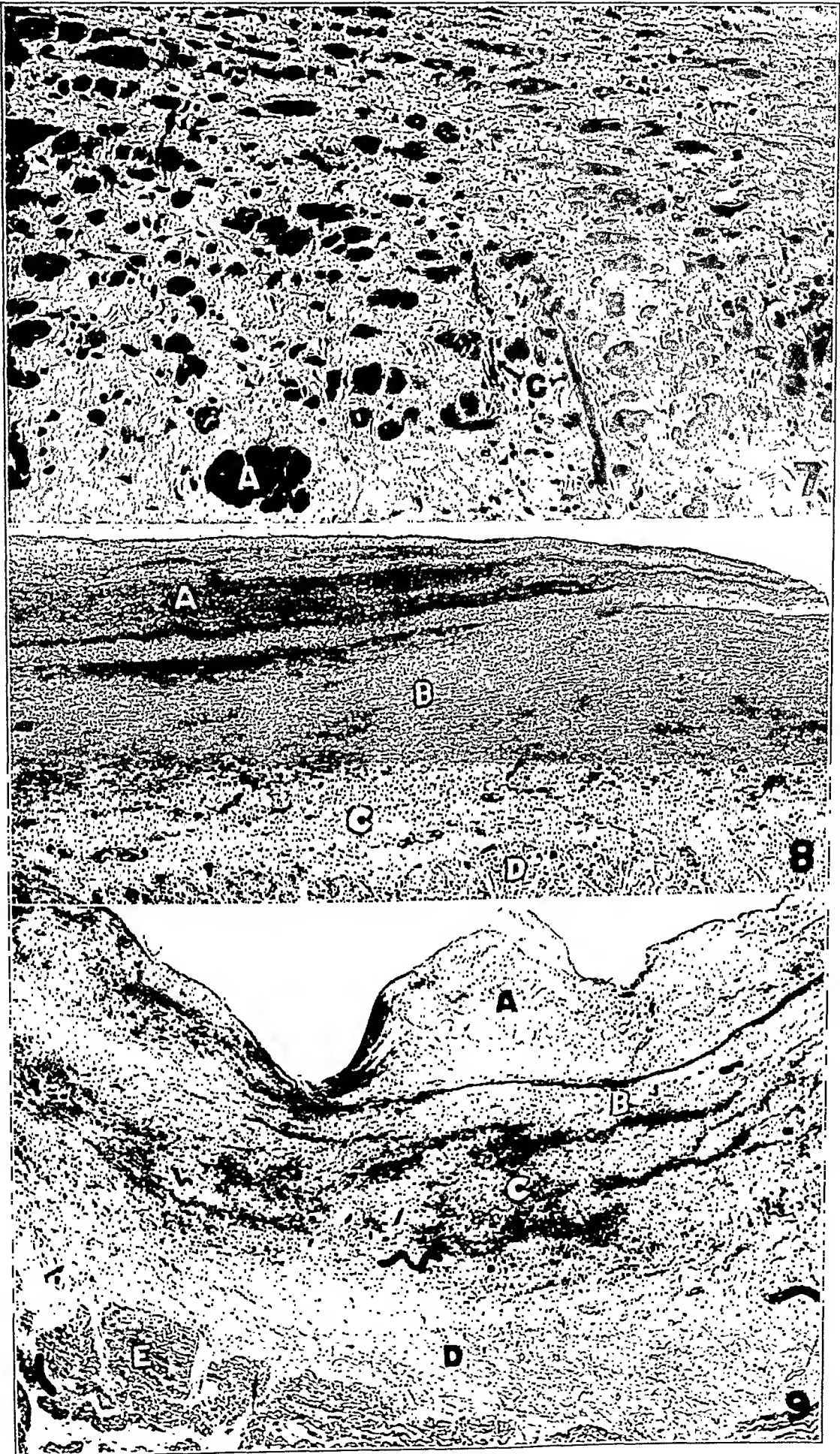


PLATE 103

FIG. 10. Left auricle from active case of rheumatic fever showing multiple reduplications. Age 14 years. Low power. Weigert's elastic and Van Gieson's connective tissue stain.

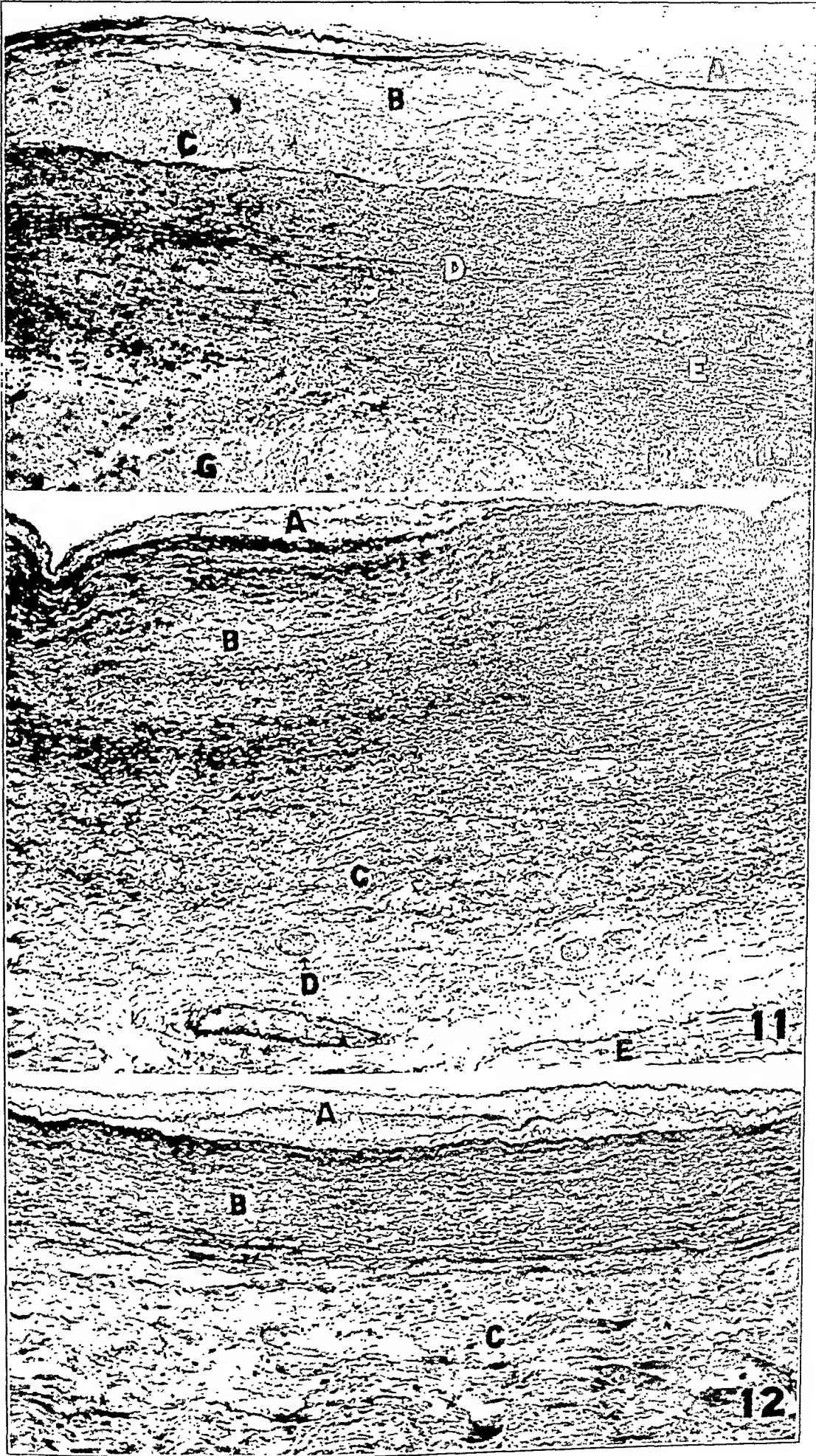
A = most recent reduplication, uncovered; B = older collagenous reduplication; C = smooth muscle component in reduplication; D = endocardium showing marked small round cell infiltration with stretching and separation of elastic fibers; E = penetrating vessels of arteriolar type, in endocardium; F = somewhat widened and infiltrated subendocardium; G = myocardium with mild interstitial infiltration.

FIG. 11. Left auricle from active case of rheumatic fever. Age 39 years. Low power. Weigert's elastic and Van Gieson's connective tissue stain.

A = collagenous covered reduplication; B = endocardium showing patchy arrangement of elastic fibers; C = markedly widened subendocardium with moderate small round cell infiltration; D = new formation of subendocardial vessels of the intimal musculo-elastic hyperplastic type; E = myocardium.

FIG. 12. Left auricle from active case of rheumatic fever showing multiple reduplications. Age 49 years. Low power. Weigert's elastic and Van Gieson's connective tissue stain.

A = multiple reduplications of endocardium. The reduplication resting on the endocardial limiting membrane is of the covered collagenous type. The innermost reduplication is of the covered embryonal type; B = endocardium; C = myocardium showing marked hypertrophy and some vascular engorgement.



THE VIRUS OF LYMPHOGRANULOMA INGUINALE *

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I. HISTORICAL REVIEW

Discovered nearly 40 years after the important group of virus diseases had been established through the pioneer work of Loeffler and Frosch in 1892 on foot and mouth disease, the virus of lymphogranuloma inguinale, or the "sixth venereal disease" (Stannus), must be regarded as one of the most recent additions to the group of pathogenic filtrable viruses. The first report of apparently successful animal transmission of the disease, known since its classical description by Trousseau in 1865 and excellently described as a clinical entity by Durand, Nicolas and Favre in 1913, was made by Darré and Dumas in 1921. Two of their 4 rabbits, 24 to 48 hours after inoculation with a few drops of pus from the inguinal bubo of a typical case of lymphogranuloma inguinale, into the anterior chamber of the eye, developed a slight iridocyclitis, which during the next 8 days progressed into a severe purulent panophthalmitis, accompanied by severe constitutional symptoms. Cultures of the exudate from the eye proved to be sterile and the animals soon completely recovered.

In the following years (1922-1924) Ravaut and his co-workers observed evanescent inguinal buboes in guinea pigs following the subcutaneous injection of pus from lymphogranuloma inguinale buboes into the region of the groin in a small percentage of their cases. They also confirmed the results obtained by Darré and

* Received for publication April 29, 1935.

Dumas in rabbits. Most investigators of this period, however, failed to produce lesions in animals and the few apparently positive results met with much adverse criticism (Favre, and Hellerström and Wassen). The diagnostic difficulties which made proper selection of true cases of lymphogranuloma inguinale for experimental purposes often impossible may, in our opinion, account for many of the discouraging results of this period, and we agree with Stannus that many of those doubtful results can be regarded as positive in the light of later experimental evidence.

With discovery of the specific diagnostic skin reaction for lymphogranuloma inguinale by Frei (1925), naturally most of the diagnostic difficulties of the disease were overcome. Shortly after, Hellerström and Wassen reported successful transmission of the disease to monkeys. In 1931 Levaditi, Ravaut, Lépine and Schoen in Paris, and Hellerström and Wassen in Stockholm, independently described a filtrable virus as the causal agent of the disease, both reports appearing in the same journal (*Compt. rend. Soc. de biol.*, 1931, 106) only a few pages apart. In the following years Levaditi and his associates, as well as the Swedish workers and many other continental scientists, continued investigations of the disease and were able to disclose numerous characteristics of the new virus.

Although the disease has been reported in the United States in its various manifestations on numerous occasions and appears to be rather common in this country, with its large negro population, the fact that comparatively little investigation had been made of its causal virus gave us the incentive to study a series of cases occurring in New Orleans. We have been able not only to isolate the virus in seven instances from excised buboes, but also to preserve its virulence by passage through animals. The 160 cases of our series, observed in the short period of 6 months (May–October), were in the majority negroes who had lived all their lives in New Orleans or its immediate vicinity, the possibility of any being infected with an imported virus strain, as observed by Wilmoth, being very remote. Our seven endemic strains of the virus have been rather critically studied and we wish in this report to record some of the data so secured. To facilitate comparison with other isolated strains these data will be presented in close correlation with a critical review of the literature.

II. SOURCES OF VIRUS STRAINS STUDIED

Table I presents data from the case history of each patient from whom we isolated virus strains used in our investigations.

TABLE I

Data on Patients From Whom Virus Strains Were Obtained

Strain No.	Patient	Sex	Race	Age	Significant clinical data	Penile sore	Frei reaction
L 20	J. J.	M	Negro	29 yrs.	Large, bilateral, tender buboes of 3 weeks duration in the inguinal region	None observed	Strongly positive
L 21	M. D.	M	Negro	17	Unilateral, firm, fluctuant bubo of 6 to 8 weeks duration adherent to the skin of the inguinal region	None observed	Strongly positive
L 24	S. J.	M	Negro	16	Unilateral, hard, tender bubo of 1 weeks duration in inguinal region	None observed	Positive
L 26	P. F.	M	White	18	Large, unilateral, fluctuant bubo of 1 months duration in inguinal region	None observed	Strongly positive
L 27	J. J.	M	Negro	16	Large, firm, unilateral mass of 4 weeks duration in inguinal region showing distinct fluctuation	None observed	Strongly positive
L 31	S. B.	M	Negro	23	Large, tender, fluctuant mass of 1 months duration in right inguinal region; burning sensation in urethra before onset of bubo	None observed	Strongly positive
L 32	W. M.	M	Negro	22	Large, fluctuant mass of 1½ months duration in inguinal region; bubo adherent to the skin of inguinal region from which drained a thick yellow exudate after surgical incision	None observed	Strongly positive

III. MANIFESTATIONS IN EXPERIMENTAL ANIMALS

Monkeys, sheep, rabbits, guinea pigs, white mice, chickens and frogs were inoculated by various routes with the isolated strains.

Characteristic lesions were observed in monkeys, sheep, guinea pigs and white mice, while rabbits, chickens and frogs proved refractory to the virus. Passage from animal to animal was performed with homologous and heterologous species of susceptible animals without any difference in the number of "takes" obtained.

(a) *Monkeys*: Two *Macacus rhesus* and 3 common marmosets (*Hepale penicillata*) were inoculated with material derived from patient J. J., (virus strain L 20). Rhesus monkey A received intracerebrally 0.1 cc. of 20 per cent inguinal gland emulsion prepared from material secured by surgical excision; rhesus monkey B received 0.5 cc. of a similar emulsion into the prepuce. The 3 marmosets were inoculated with 0.1 cc. of 20 per cent brain emulsion obtained from a mouse injected intracerebrally 8 days previously with 20 per cent inguinal gland emulsion.

The rhesus monkeys during 6 months of observation did not show any local or general symptoms of disease. The 3 marmosets began to show signs of illness 5 to 9 days after inoculation. The first characteristic symptoms consisted of loss of appetite and general muscular weakness, soon followed by increased muscular irritability and epileptiform convulsions. One to 3 days after onset of symptoms paralysis of the extremities was noted, usually appearing first in the hind legs. Finally, the monkeys became comatose and died 7, 9 and 13 days, respectively, after inoculation. Autopsy showed essentially slight hyperemia of the brain and meninges.

Microscopic study of sections from the brain and cord with their meninges showed dense meningeal infiltration with an increased cellular exudate. The cell types were mostly young large lymphocytes and large mononuclear cells (monocytes), neutrophilic leukocytes and plasma cells being distinctly in the minority, but more apparent however in the animal that died 7 days after inoculation. The meninges of the convex side of the brain and those at the base were equally affected, the exudate being especially marked in the deep sulci. The meninges of the ventricles showed similar infiltration. The nervous elements of the brain and of the spinal cord showed no appreciable changes. Some of the ganglia cells near the sites of infiltration were slightly pyknotic and appeared somewhat shrunken, but no absolute evidence of degeneration could be observed. The Virchow-Robin spaces of a large number of vessels of the brain and of the spinal cord showed dense perivascular, collar-

like infiltration with lymphocytes and large mononuclear cells. In a future report these pathological findings will be described in more detail and compared with changes evoked by other filtrable viruses.

Antigens prepared after Frei's technic with 20 per cent infected brain emulsion in sterile saline solution gave positive skin reactions in patients afflicted with lymphogranuloma inguinale. The advantages of this method of preparing the antigen for the diagnostic skin test have been emphasized (von Haam and Lichtenstein) on previous occasions.

Intracerebral inoculation of 20 per cent saline emulsions of infected monkey brains in mice produced typical lesions. Transmission of the virus to mice could also be effected by intracerebral inoculation of heart's blood and 20 per cent spleen emulsion from infected monkeys, but not by similar injections of liver and kidney emulsions.

Our observations on the manifestation of the virus of lymphogranuloma inguinale in the monkey agree, except in few instances, with the experiences of the European workers. The difference of susceptibility of the various species of monkeys, as stressed first by Hellerström and Wassen, and Levaditi and co-workers, was well demonstrated in our small series of animals, both rhesus monkeys remaining resistant to the virus. Similarly, Findlay succeeded in infecting only 3 out of 10 rhesus monkeys.

Thus far none of our infected monkeys have recovered after showing signs of the disease, and we were unable to study serum protective or virucidal action of such serums as has been done by Findlay, Levaditi and co-workers. The presence of the lymphogranuloma inguinale virus in other organs than the brain following intracerebral inoculation had been previously demonstrated by Hellerström and Wassen, and Levaditi and co-workers, who were able to transmit the disease with heart's blood and splenic emulsions obtained from intracerebrally infected animals. Levaditi also emphasized the possible passage from monkey to mouse and again to monkey. With us this has proved an excellent method of preventing "autosterilization of the virus" observed after continuous passage through monkeys or kittens.

None of our animals showed degeneration of the posterior tracts of the spinal column with demyelination of the nerve fibers and axis cylinder changes, as reported by Jonesco-Mihaiesti and co-

workers. Levaditi and Mollaret in a recent publication believe that the changes reported by these Roumanian workers are the results of spontaneous lesions described by him and others some years ago.

Positive Frei reactions with brain emulsions of infected monkeys were obtained by Hellerström and Wassen, Cohn and Kleeberg, and the pathogenicity of unheated brain emulsions for man was demonstrated in a single experiment by Levaditi and his co-workers. The interesting observation of Bonne and co-workers in Dutch East India that heated monkey brain antigen produced in three volunteers typical axillary paradenitis is believed by Stannus to be due to faulty technic in antigen preparation and calls for more confirmation. This we have not been able to do.

(b) *White Mice*: Because of the enthusiastic reports of Findlay and Wassen, we employed white mice extensively in transmission experiments. In the experiments herein reported we made use of 345 mice, 85 serving as controls. All animals came from a well known source and were carefully observed in isolation cages before use. Intracerebral injections were performed with a very fine short needle (26 gauge, $\frac{1}{4}$ inch), the material injected (inguinal bubo pus from human cases or 20 per cent infected brain emulsion in saline) never exceeding 0.01 cc. For hours after injection most animals behaved normally, the incidence of serious traumatization of brain substance by injection being negligible. All seven virus strains were used in mice experiments and were mostly kept virulent by means of numerous animal passages. Strain L 20 was carried at the time of presentation of this report through 17 passages; strain L 26 through 11 passages; strain L 27 through 11 passages; strain L 31 through 9 passages; and strain L 32 through 7 passages. Strains L 21 and L 24 lost their pathogenicity for mice after the 3rd passage. With strain L 20 we attempted to determine the most advantageous interval between inoculations in order to maintain optimum virus virulence. Accordingly, groups of mice were inoculated at weekly, biweekly, and monthly intervals and the comparative strength of the virus estimated from the percentage of "takes," the time of appearance and severity of clinical symptoms and the histological pictures of the lesion produced. Weekly or biweekly intervals were found best for virulence-sustaining inoculation, monthly intervals generally leading to decrease of virus virulence. This can be explained by the fact that only more resistant or less

infected animals survive as long as 4 weeks after inoculation with the virus; subsequent transmissions from such animals will then be made with brain material containing less virus or more immunizing substances. We routinely use biweekly intracerebral mouse inoculations in carrying our strains.

The incubation period in white mice was characterized by wide individual variations. Some of the animals showed symptoms as early as 4 days after intracerebral injection, while others were apparently perfectly well at the time they were killed for scheduled passage of the virus to other animals. Animals that died 1 to 3 days following injection were regularly discarded, as in such cases trauma from the injection played too large a factor to allow correct conclusions. In the majority of such cases the animals had shown severe shock following injection, with paralysis of one or more extremities occurring for several hours.

The first symptoms attributable to the virus in white mice following intracerebral injection were weakness, loss of appetite and roughened appearance of the fur; the animals moved slowly and listlessly and lost weight. Later, nervous symptoms became manifest. These consisted of muscular weakness with paralysis of the extremities, especially the hind legs; epileptiform movements and sometimes chronic convulsions occurring in short attacks. The typical "encephalitis position," as described by Fischl and Schaefer in experimental herpes encephalitis in mice, was observed in several instances. Toward the later stages severe unilateral or bilateral conjunctivitis was usually noted, with hemorrhagic exudate covering the cornea. Animals showing this symptom complex usually died in 3 to 5 days after its onset; none recovered spontaneously.

Autopsy and histological studies of infected mouse brains showed lesions similar to those described in the brains of infected monkeys. In all infected mice the typical histological changes could be observed as early as 4 days after intracerebral inoculation, being independent of clinical symptoms.

The microscopic picture of the lesion was that of an extremely cellular exudate consisting of lymphocytes and large endothelial cells around the vessels of the meninges. In some few cases more fibrinous exudate and a larger number of neutrophilic leukocytes were noted than were observed in the brains of similarly infected monkeys. In animals killed 1 month after inoculation, without the

advent of nervous symptoms, the infiltration was limited to a more or less circumscribed collection of cells, mostly small lymphocytes, around the larger vessels of the meninges — so-called "lymphomas."

Except for the very weak virus strains (L 21 and L 24), we were able indefinitely to reproduce the same type of lesions by repeated intracerebral inoculations of 20 per cent infected brain emulsions. With such infected mouse brain emulsions we were also able to produce typical lesions in monkeys, sheep and guinea pigs, but not in chickens or frogs.

The antigenic value of infected mouse brain emulsions, as determined by the Frei test, was generally as high as that observed with emulsions of infected monkey brains. Spleen and kidney emulsions of intracerebrally infected mice also gave positive Frei reactions in patients with lymphogranuloma inguinale; such reactions, however, were much weaker than the ones produced with brain emulsion from the same animals.

In Findlay's mice experiments considerable variation in incubation time was noted. For his 586 mice that died of fatal meningo-encephalitis produced by intracerebral inoculation of lymphogranuloma inguinale virus, the incubation period was between 5 and 94 days, with an average of 34 days. Contrary to this observation, Grace and Suskind report an incubation period of 2, 4 and 7 days in their passage experiments with a virus strain isolated in New York. Our experience in this respect agrees more with that of Findlay. However, with many of our mice no signs of disease were noticeable when the animals were killed for routine transmission of virus 2 weeks after infection. The difference between the results obtained by Grace and Suskind, and Findlay and ourselves may be caused by varying virulence of the viruses used, a factor stressed by Levaditi and his co-workers. Another contributing factor may be the dosage of inoculated material. In Findlay's report no data concerning dosage are given. Grace and Suskind injected 0.03 cc. of 20 per cent and 40 per cent brain emulsions, which is considerably more than the dosage used by us. Although their control animals did not show any detrimental effect from this dosage, it is possible that the higher degree of traumatization of the brain accelerated the effects of the virus. Such combined effects of brain trauma and remote inoculation with virus of lymphogranuloma inguinale have been reported by Findlay, who obtained localization of the virus in the

brain after intraperitoneal injection of the infectious material, the brain having been previously traumatized by injection of sterile starch solution. In the course of our transmission experiments we received the impression that the virulence of some of our strains for mice increased with successive passage; our observations, however, do not point as strongly in this direction as do those of Grace and Suskind.

The histological picture of the experimental meningo-encephalitis produced by inoculation of lymphogranuloma inguinale material must be differentiated from the picture of spontaneous encephalitis in white mice, as described in this country by Cowdry and Nicholson. In this condition infiltration of the meninges, the perivascular spaces of the brain and the subependymal areas with round cells is also seen; the type of infiltration, however, is more focal and the cell type mostly the small lymphocyte. Cowdry and Nicholson, who described 25 cases of spontaneous encephalitis among 141 stock mice, emphasized that the animals showed no symptoms of disease and could not be differentiated from healthy ones. They observed a protozoan-like parasite similar to the *Encephalitozoön cuniculi* (Levaditi) in brain sections of these animals. In reporting experiments with white mice Levaditi and co-workers emphasized the fact that the virus of lymphogranuloma inguinale, similar to *Encephalitozoön cuniculi* or the virus of recurrent fever, could remain in the central nervous system of white mice without apparently harming the animals. We have encountered spontaneous encephalitis in some of our local mice strains and wish to point to the advisability of using strains of mice free from or usually quite resistant to epizootic encephalitis in studying neurotropic viruses. As additional precaution, we considered positive "takes" only such animals whose brain emulsions were able to elicit unmistakably positive Frei reactions in humans suffering from lymphogranuloma inguinale.

(c) *Guinea Pigs*: Many authors report negative or doubtful results in transmission experiments with guinea pigs. In contrast to the many discouraging reports, however, Meyer, Rosenfeld and Anders recorded nearly 100 per cent success in their guinea pig inoculation experiments. They apparently observed typical lesions produced by the virus not only in the regional lymph glands but also in the mesenteric glands, lungs, spleen and liver. Nicolau,

likewise, reported such generalized infection of guinea pigs with the virus. He and Findlay, however, sound a warning that spontaneous lesions in guinea pigs caused by infection with *Pasteurella pseudotuberculosis* may be mistaken for lesions of experimental lymphogranuloma inguinale. In these views we fully concur as a result of our own experiences.

Fourteen guinea pigs were inoculated with infected mouse brain emulsions (strain L 20). Each animal received 0.1 cc. of 20 per cent organ emulsion subcutaneously in both inguinal regions. The slight infiltration following injection disappeared completely after a day. After intervals of from 4 to 8 days, 5 of the inoculated animals developed palpable enlargement of the inguinal glands. These animals were sacrificed and their inguinal regions exposed. The lymph glands were markedly enlarged and of a dark reddish gray color. Surfaces of the swollen glands revealed by sectioning showed small yellow areas suggesting microscopic abscesses. The periglandular connective tissue was injected, the lymph vessels clearly visible. The disease process was always localized in the inguinal glands and no generalized infection, as described by some continental authors, could be observed. Microscopic pictures of the glands showed marked proliferation of the endothelial cells with some giant cells present, together with infiltration with polymorphonuclear leukocytes in the form of small cell collections resembling the microscopic abscesses so typical of inguinal buboes seen in human lymphogranuloma inguinale. Our few attempts at transmitting the disease by means of glands of such infected guinea pigs to other animals have so far failed.

(d) *Other Animals*: Although greatest success is obtained in transmission experiments by the use of monkeys and white mice, numerous other species of animals can be infected with the virus of lymphogranuloma inguinale. Freund and Reiss, and Chevallier and his co-workers report successful transmission to rabbits by intracerebral inoculations. On the other hand, Findlay obtained negative results with 16 rabbits injected subcutaneously in the groin region and similar negative results following infection by the intracerebral route. He concludes, however, that the virus may remain virulent in the central nervous system of rabbits for at least 10 days. Levaditi, Ravaut, Schoen and Vaisman successfully infected cats; Nicolau and Findlay, dogs. In the brains of field moles the virus

retains its virulence for white mice for 30 days without apparently harming the intermediate host.

We inoculated 2 sheep (mother and young) intracerebrally with 0.5 cc. of a 20 per cent pooled brain emulsion of infected mice. The lamb died after 5 days. Histological studies showed typical meningo-encephalitis. Twelve young chickens (white Leghorn) inoculated intraperitoneally and intracerebrally with a very infectious pooled mouse brain emulsion remained symptomless with no demonstrable histological lesions. White mice, however, injected intracerebrally with such infected chicken brain emulsions showed typical meningeal reactions, while control mice injected with normal chicken brain emulsions showed no clinical or histopathological changes whatsoever. This shows, as previously pointed out by Findlay, that the virus is able to survive in the chicken brain for some time without losing its virulence. Eight frogs (*Rana catesbiana* and *Rana pipiens*) inoculated with infected mouse brain emulsions showed no symptoms of disease or histopathological changes in their central nervous systems. Mice inoculated with infected frog brain emulsions remained symptomless and showed no histological changes.

IV. CULTIVATION OF THE VIRUS

All early attempts at cultivating the virus of lymphogranuloma inguinale failed (Stannus). In 1931 Langer, in discussing a paper by Levaditi on this subject, reported apparently successful cultivation of the virus in symbiosis with cell cultures. Of 19 guinea pigs inoculated by him with the second to fifth subcultures of the virus, 10 showed the typical lesions of human lymphogranuloma inguinale, in 4 the histological lesions were "doubtful," and 6 showed no changes or only a non-specific inflammatory reaction. Seven control animals showed no histological changes or only non-specific inflammatory changes. The virus cultures did not give positive skin reactions with patients suffering from the disease. Langer conceded that the latter factor and the uncertainty concerning the histopathology of lymphogranuloma inguinale in the guinea pig considerably restricted the scientific value of his results. In 1935 Tamura reported cultivating the virus of lymphogranuloma inguinale in the medium devised by Maitland and co-workers for the cultivation of vaccinia. From 0.02 cc. to 0.03 cc. of diluted pus secured from an

inguinal bubo inoculated into 7 cc. of Tyrode solution containing a small piece of fresh liver or kidney from guinea pigs produced, after incubation from 36 to 48 hours, a peculiar cloudiness of the medium which was transmissible and could be carried on through as many as twenty-four subcultures. With such heated cultures Tamura was successful in eliciting positive Frei reactions in patients suffering from lymphogranuloma inguinale.

Applying Tamura's method, 0.1 cc. of 20 per cent infected mouse brain emulsions were placed in sterile test tubes containing 10 cc. of Tyrode solution and approximately one-third of the kidney of a rabbit starved for 24 hours before death. The medium was usually prepared the day preceding inoculation and incubated for 12 hours to insure sterility. Two to 3 days after inoculation a distinct cloudiness appeared in the tubes inoculated with infected material, while control tubes remained clear. From the second and the third subcultures mice were inoculated intracerebrally with 0.01 cc. of the cloudy fluid. They developed the characteristic histopathological changes previously described. Mice inoculated with material from control tubes 8 days after inoculation showed no signs of any meningeal reaction. Examination of the cloudy Smith-Noguchi-Maitland tubes failed to show bacterial growth. The dilution of the primary mouse virus in the second subculture was 1:10,000. We offer this result as preliminary confirmation of Tamura's work, believing that the characteristic meningo-encephalitis produced in white mice after inoculation of culture material must be regarded as more convincing proof of cultivation of the virus of lymphogranuloma inguinale after Tamura's method than his own evidence derived from guinea pig experimentation.

V. SUMMARY AND CONCLUSIONS

1. Seven endemic strains of the virus of lymphogranuloma inguinale have been isolated and transmitted to animals.
2. Intracerebral inoculation of infectious material produced a typical meningo-encephalitis in the common marmoset while the rhesus monkey proved resistant to such inoculations.
3. The virus could readily be transmitted to white mice, biweekly inoculations allowing upkeep of its maximal virulence.
4. Brain emulsions of infected monkeys and mice act as excellent

stable and sensitive antigens for the specific diagnostic intradermal reaction of Frei.

5. Twenty-eight per cent of infected guinea pigs showed enlargement of the regional lymph glands with histological lesions consistent with the disease.

6. Experiments with sheep, chickens and frogs indicate that the virus can infect sheep; that its virulence can be preserved in the brains of chickens; and that frogs cannot be infected.

7. Cultivation of the virus after the method of Tamura was possibly confirmed.

8. The virus of lymphogranuloma inguinale, as encountered endemically in the poorer negro population of Louisiana, shows rather identical behavior concerning animal transmission as the virus strains studied in other parts of the United States and abroad.

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DESCRIPTION OF PLATES

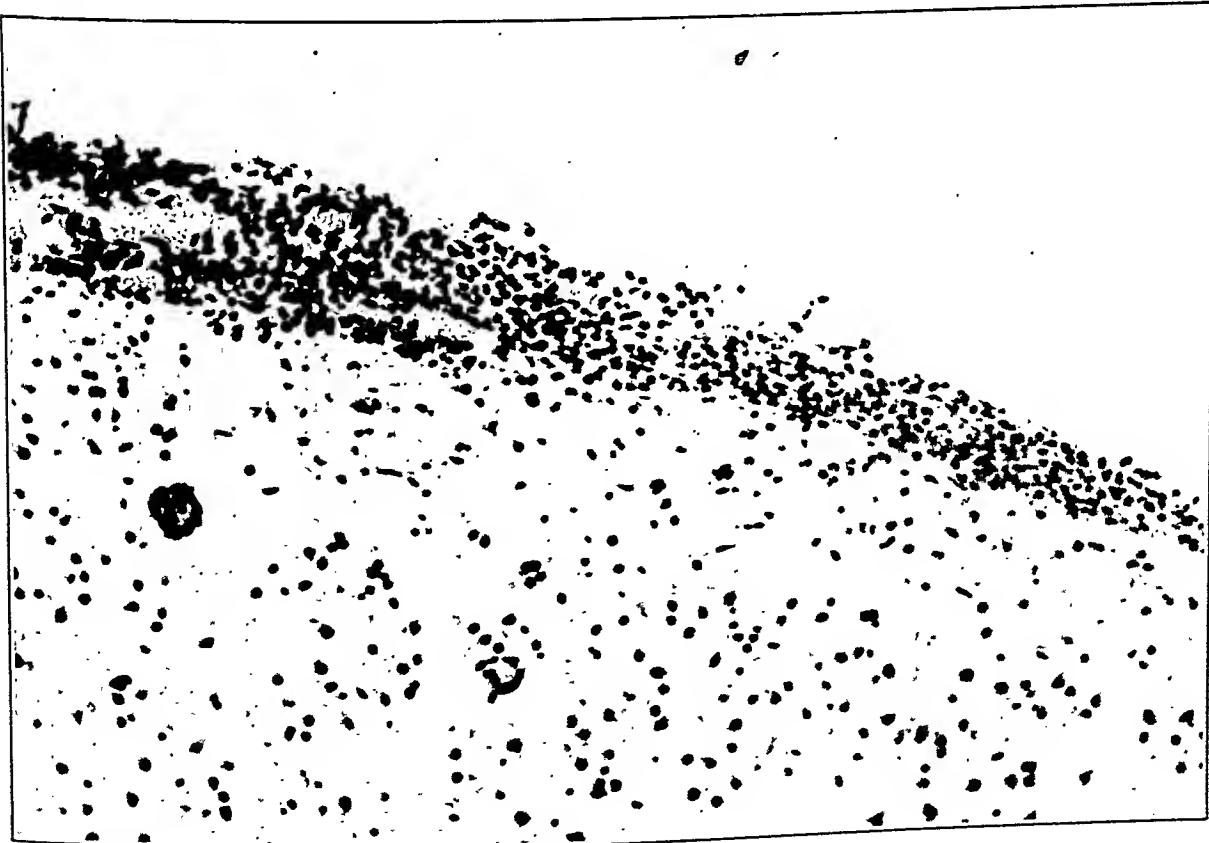
PLATE 104

FIG. 1. Intracerebrally infected monkey (*Hepale penicillata*). Complete paralysis with convulsions 9 days after inoculation.

FIG. 2. Meningo encephalitis in infected monkey (*Hepale penicillata*). Dense infiltration of meninges with small round cells. Hematoxylin-eosin stain. $\times 120$.



1

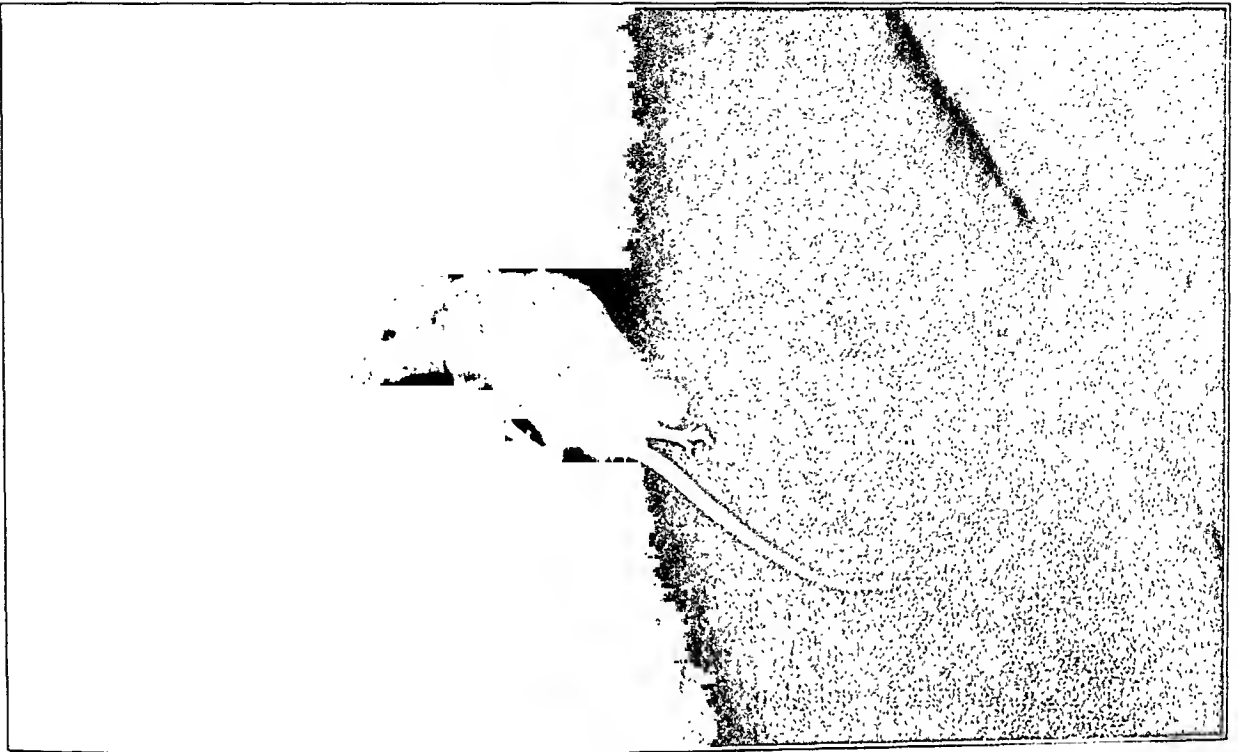


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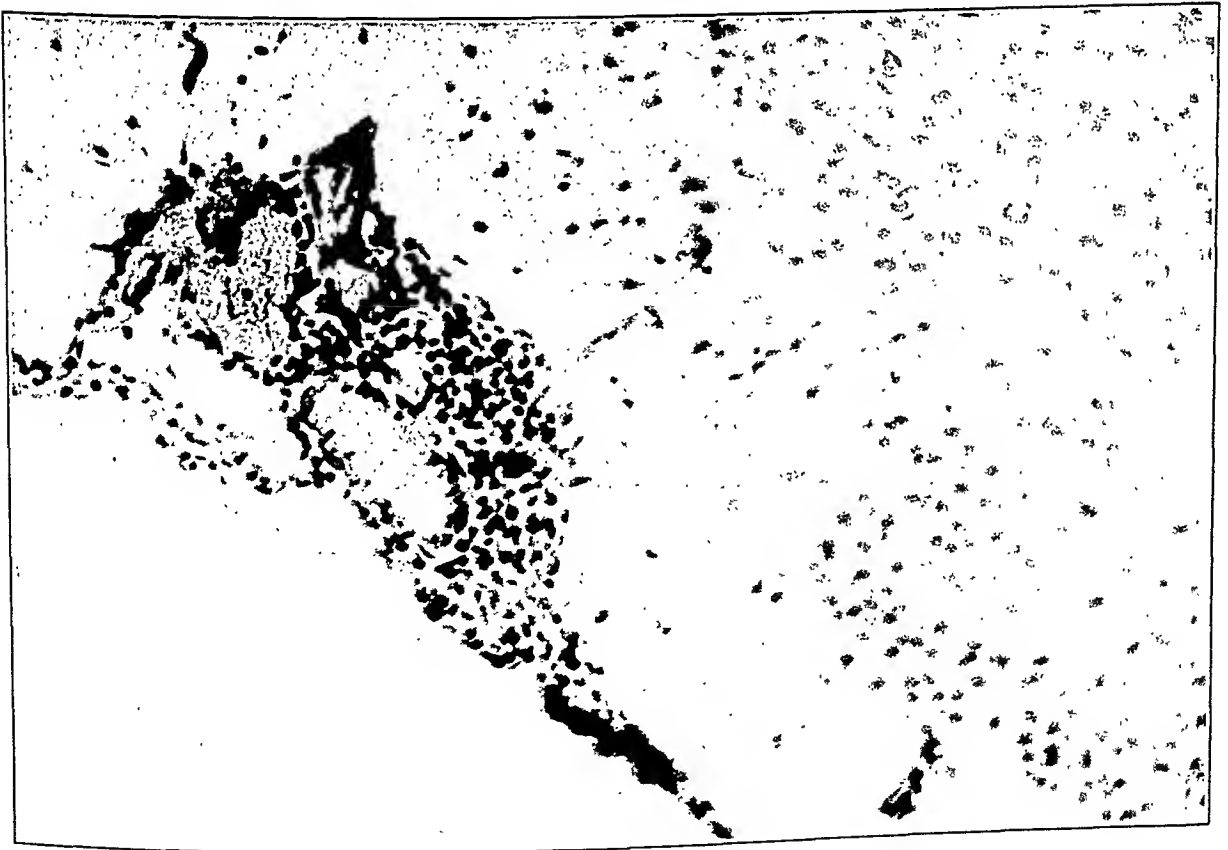
PLATE 105

FIG. 3. Intracerebrally infected white mouse. Severe paralysis with convulsions 11 days after inoculation.

FIG. 4. Meningo-encephalitis in white mouse. Focal infiltration of meninges with round cells ("lymphoma"). Hematoxylin-eosin stain. $\times 120$.



3

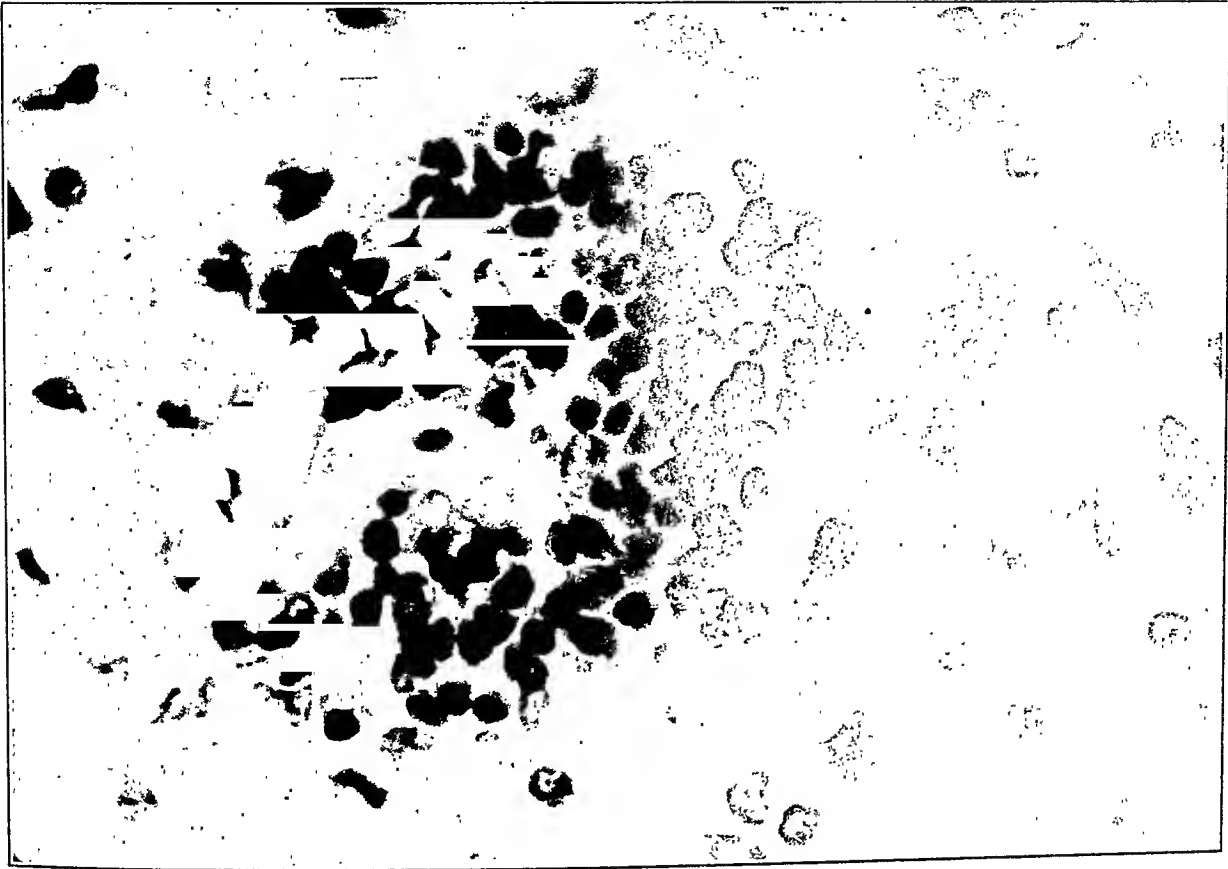


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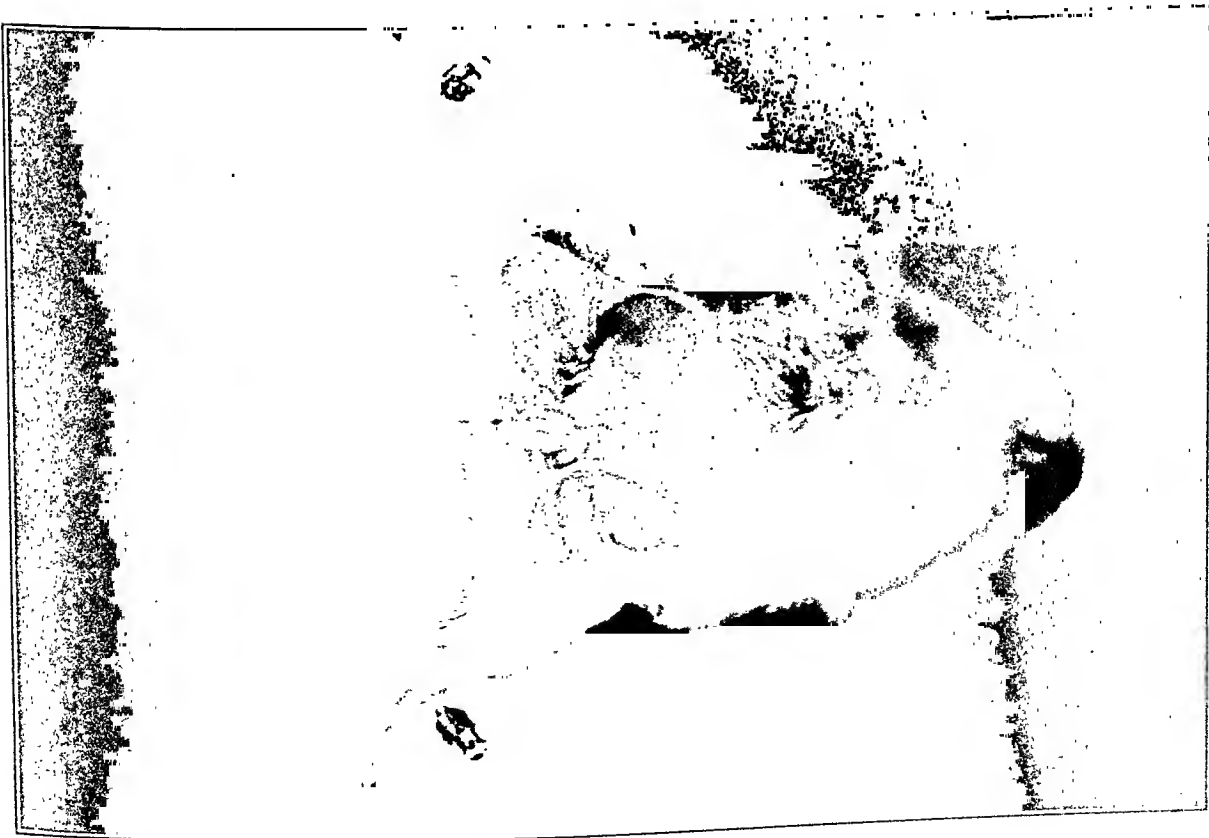
PLATE 106

FIG. 5. Meningo-encephalitis in white mouse. Perivascular round cell infiltration of Virchow-Robin space of the brain. Hematoxylin-eosin stain. $\times 450$.

FIG. 6. Subcutaneously infected guinea pig. Enlargement of regional glands 8 days after inoculation.



5



6

PLATE 107

FIG. 7. Acute bubo in guinea pig. Numerous endothelial cells and polymorphonuclear leukocytes. Hematoxylin-eosin stain. $\times 120$.

FIG. 8. Cultivation of the virus after Tamura. Note the cloudiness of the inoculated Maitland media.



7



8

RHABDOMYOSARCOMA OF THE PROSTATE *

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(From the Laboratory Service of the Walter Reed General Hospital, Washington, D. C.)

Tumors arising from striated muscle are rare. When such tumors are composed of well differentiated striped muscle the growth is classified as a rhabdomyoma. Rhabdomyomas are usually benign, sharply circumscribed and of developmental origin. Malignant rhabdomyomas (rhabdomyosarcomas) are composed of undifferentiated striped muscle. Atypical striped muscle cells may form a part of malignant teratoid tumors arising in the kidney, testis, ovary and sacral region. Approximately 50 per cent of the pure type of rhabdomyosarcomas occur in the genito-urinary tract.

Like other malignant neoplasms arising from mesenchyme, rhabdomyosarcomas metastasize by blood stream and regional lymphatics and give early lung and bone involvement. The prostate presents excellent opportunity for blood stream seeding by way of the venous plexi beneath the capsule. The malignancy of rhabdomyosarcoma, as of all newgrowths, is in inverse ratio to the degree of differentiation of the tumor cells.

Malignant rhabdomyomas primary in the prostate are rare. Ewing¹ cites 3 cases, all occurring in young adults. Stout² mentions 1 case of malignant rhabdomyoma reported by Kretschmer. Kretschmer³ in his article, "Sarcoma of the Prostate," presents 1 case of rhabdomyosarcoma, age 31 years. Examination of tissue removed at operation was diagnosed as rhabdomyosarcoma by Dr. A. S. Warthin, of the University of Michigan.

De Rom and Thomas⁴ state that 36 per cent of prostatic sarcomas are of the round cell type and 20 per cent of the fusiform cell type; the 44 per cent remaining include myxosarcomas, lymphosarcomas, angiosarcomas, fibrosarcomas, leiomyosarcomas and rhabdomyosarcomas. They quote Ewing as follows: "The only well defined variety of prostatic sarcoma is the rhabdomyosarcoma." In the case of rhabdomyosarcoma reported the patient was 23 years of age. The article is well illustrated.

* Received for publication April 15, 1935.

Culver⁵ collected 59 cases of prostatic sarcoma from the literature and added 17 more. These 76 cases include 24 of the round cell type, 17 of the spindle cell type, 8 myxosarcomas, 5 lymphosarcomas, 4 rhabdomyosarcomas, 3 angiosarcomas, the remainder being undifferentiated. The case he reports is of the large round cell type.

Greig⁶ reported a case of rhabdomyosarcoma of the prostate in a child aged 4 years. Initial incontinence was unaccompanied by pain, but pain developed later during micturition. A suprapubic cystostomy was performed and "a large fungating tumor, size of Tangerine orange, found." The patient died 10 days after operation. The microscopic diagnosis was rhabdomyosarcoma.

Katzmann⁷ published an article on malignant rhabdomyoma of the prostate in a child. He cited 30 cases of prostatic sarcoma reported up to the time of his article, among which there were 3 cases of malignant rhabdomyoma. His conclusion is that while sarcoma of various types may occur at any age, rhabdomyosarcomas are confined to childhood. This conclusion is not borne out by the findings of other observers.

The records of the Walter Reed General Hospital* include one sarcoma of the prostate occurring in a white male aged 31 years. Microscopic examination of the tissue removed at operation showed a spindle cell tumor, the cells arranged in interlacing bundles. The diagnosis was malignant fibrosarcoma.

References to prostatic sarcoma, type not classified, are numerous. All articles on this subject stress the rarity of the condition, its occurrence during youth and the rapidity of the course. A prostatic sarcoma has a soft, balloon-like feel. Tenderness is frequently absent. Deaver⁸ very aptly states that by the time the patient seeks medical advice the tumor will have reached considerable size, almost completely filling the pelvis. The bladder is pushed up and forward, the bladder wall not invaded. Cystoscopic examination is only valuable in demonstrating an extravesical mass with elevation and distortion of the trigonal area. Boyd⁹ brings out the difficulty in differentiating sarcoma from a highly anaplastic carcinoma. In the differential diagnosis between sarcoma and carcinoma of the prostate it is important to stress the wide difference in age incidence, rapidity and size of growth, and degrees of firmness and tenderness on palpation. The prostatic carcinomas are hard and nodular; the

* Walter Reed General Hospital, Washington, D. C., Reg. No. 53695.

sarcomas are larger and elastic to the touch. Residual urine is usual in prostatic carcinoma and not generally present in sarcoma.¹⁰ Carcinoma of the prostate is frequently encountered while sarcoma is relatively rare. The bone metastases in carcinoma of the prostate are primarily of the pelvic girdle and are of the osteoplastic type; in sarcoma of the prostate the bone metastases are general and are of the osteolytic type. Multiple pulmonary metastases suggest sarcoma.

The prognosis and treatment of sarcoma of the prostate link themselves with the degree of anaplasia, applicable to malignant neoplasms in general.

Briefly reviewing the anatomy of the prostate^{11,12}: the prostate normally measures 4 cm. transversely at base, 2 cm. in its antero-posterior diameter, and 3 cm. in its vertical diameter. Its weight is about 8 gm. It is located around the commencement of the male urethra, below the urinary bladder and presents thirty to fifty compound tubulo-alveolar glands located in the lateral lobes. These glands empty by from sixteen to thirty-two excretory ducts, opening into the urethra along the grooves on either side of the colliculus seminalis. The prostatic acini have no distinct basement membrane. The interstitial tissue is abundant and represents more than one-half the entire mass of the organ. The interstitial tissue is composed of connective tissue, elastic network and abundant smooth muscle arranged in strands. That portion of the prostate lying behind the pubic bone is represented by a wedge-shaped section, whose apex is directed toward the anterior wall of the prostatic urethra. This wedge-shaped section lies between the two lateral lobes and is composed of dense connective tissue traversed by bands of striped muscle fibers. The fibromuscular stroma of the prostate includes large blood vessels and large sympathetic trunks and ganglia. The veins form a plexus beneath the capsule. A primary newgrowth of the prostate may, therefore, arise from any of the above structures, starting from tissue derived from any of the three cell layers of the primitive embryo.

The following case is reported as being typical of prostatic sarcoma. The age of the patient, the subjective and objective clinical findings, the clinical course, termination and autopsy findings, all are true to this type of malignancy of the prostate. The microscopical examinations further differentiated the neoplastic process as a malignant rhabdomyoma.

REPORT OF CASE

Clinical History: G. R. M., a white male carpenter, 26 years of age, born in Indiana, was admitted to the Letterman General Hospital Aug. 12, 1933. The family history was irrelevant. He had the "usual childhood diseases" and typhoid fever during early adolescence; denied venereal infection and the use of alcohol.

Present Illness: Two weeks prior to admission to the hospital the patient, a member of the Civilian Conservation Corps, noticed a burning sensation in the urethra. He became unable to void and was catheterized. It was only necessary to catheterize once, as after passage of the catheter the patient developed frequency of urination and felt as though he were improving. At this time he noticed a bloody discharge from the external meatus. He was transferred to the Letterman General Hospital. On admission he complained of no subjective symptoms.

Physical Examination: This revealed a greatly enlarged prostate, soft and compressible. The diagnosis of the ward surgeon was prostatic abscess complicated by rupture into the ampulla of the rectum. A blood culture was sterile.

Laboratory Data: Blood chemistry showed urea nitrogen 96 mg., and creatinine 2 mg. per 100 cc. Urine examinations showed a heavy trace of albumin, many pus and red blood cells. Wassermann negative.

Course of Illness: On September 7th cystotomy was performed. The prostate was found to be extremely large and soft, protruding into the lumen of the bladder over the trigonal area. An attempt was made to find the suspected abscess without result. The patient died Sept. 11, 1933.

The course of the case, from the onset of the first subjective symptom (urethral burning) until death, was only 44 days. The autopsy findings are so generalized and massive that it is not fair to assume that the exploratory cystotomy hastened death.

AUTOPSY FINDINGS *

The body is that of a white male, 26 years of age, 165 cm. in length, weighing approximately 50 kg. (110 pounds). Moderate emaciation is present. There is massive neoplastic infiltration of the left, deep cervical lymph nodes which push the larynx and trachea to the right. The thyroid weighs 23 gm. and presents one small metastasis. The posterior bronchial and hilum nodes, bilateral, are massively infiltrated. Both lungs present sharply outlined nodules, varying in size from 0.5 to 2 cm. in diameter, located beneath the pleural surfaces of all lobes. The outer surfaces of these nodules are flattened, smooth and of mushroom shape. The cut surfaces of the lungs present many metastatic nodules located throughout the cortical portion of all lobes. The heart is free from metastatic involvement. There is no peritoneal involvement. The liver weighs 2324

* Autopsy No. 1367, Letterman General Hospital, San Francisco, Calif.

gm.; its surface is studded by circular, slightly raised areas from 0.5 to 2 cm. in diameter. The cut surface of the liver presents a few spherical nodules. As no X-rays had been taken, only the more massive bone metastases are noted, osteolytic in type, including two of the calvarium and one replacing the body of the twelfth dorsal vertebra. The kidneys show the picture of a chronic, suppurative (ascending) pyelonephritis, more advanced in the left kidney. The spleen weighs 342 gm. and is of the septic type.

The *urinary bladder* shows an operative incision through the antero-inferior wall closed by interrupted catgut sutures, excepting the upper angle which admits a rubber tissue drain into the bladder lumen. The bladder wall is contracted, the mucosa presenting a hemorrhagic, ulcerative reaction. The ureteral orifices are obscured by hemorrhage and edema of the surrounding mucosa. The trigone is replaced by a large, soft, pale pinkish gray mass, the summit of which shows a transverse laceration (made at the time of exploratory cystotomy).

The *prostate* is 11 cm. in diameter. The anterior and lateral aspects of the prostatic mass are relatively firm and their cut surfaces are pinkish gray, smooth and homogeneous. The posterior aspect is necrotic, reduced to a grayish brown substance showing central liquefaction. The *newgrowth* replaces the entire prostate and has destroyed the seminal vesicles and ampullae of the vasa deferentia, and infiltrates the rectovesical space, fungating through the adjacent wall of the rectum. The rectum wall presents a circular orifice with a necrotic edge. The prostatic urethra is elongated, its lining surface necrobiotic, the normal markings obliterated. The *lymph nodes* lying upon the external iliac vessels are greatly enlarged, on the right side forming a globular mass 4 by 6 cm. The infiltrated nodes are soft and necrobiotic.

Microscopic Appearance of the Newgrowth: The tumor is extremely cellular, composed of spindle cells with large oval nuclei and small nucleoli. The cell outlines are poorly marked. Mitotic figures are numerous and atypical. There are many areas of hemorrhage. In the lung the newgrowth fills the lumens of the aveoli and extends into the lumens of the veins. The rapidity of this growth is shown in the liver metastases where the death of the invaded parenchyma does not keep pace with the neoplastic infiltration. Careful study of the cell morphology of the newgrowth presents: (a) scattered cell nests,

composed of more highly differentiated cells presenting varying amounts of acidophilic cytoplasm with sharp cell outlines; (b) spindle cells, the acidophilic cytoplasm of which shows both cross and longitudinal striae; (c) large, round, oval and giant cells presenting well differentiated striae, both cross and longitudinal, massed in the periphery of the cell body; (d) large cells showing an unstained perinuclear zone crossed by fibrils, producing a spider-like appearance. The illustrations reveal the minute architecture of the more highly differentiated cells, malignant variants of striped muscle.

CONCLUSIONS

Prostatic sarcoma is rare but its possibility must be considered when there is prostatic enlargement, even in adult life. The differential diagnosis between sarcoma and carcinoma of the prostate is made by rather wide differences: (a) in age incidence; (b) in rapidity of the growth; (c) in consistence of the growth; (d) by the absence or presence of local tenderness; and (e) by the location and character of the bone metastases.

Among the various types of sarcoma encountered, rhabdomyosarcoma offers cell detail clearly denoting the parent cell from which the growth originated.

NOTE: I desire to express to the Curator of the Army Medical Museum my appreciation of the splendid results obtained in taking the photomicrographs.

Dr. Arthur Purdy Stout of Columbia University was kind enough to express his opinion regarding this case.

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DESCRIPTION OF PLATES

PLATE 108

FIG. 1. Lung metastasis showing a fusiform cell presenting cross and longitudinal striae and larger cells showing perinuclear rarefaction. Hematoxylin-eosin. $\times 2160$.



1

Foucar

Rhabdomyosarcoma of Prostate

PLATE 109

FIG. 2. Lung metastasis showing giant cells with deep rims of striated cytoplasm and larger giant cells of spider type. Hematoxylin-eosin. $\times 2160$.



AN ANENCEPHALIC MONSTER WITH "RHINODYMIE" AND OTHER ANOMALIES *

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This report concerns an anencephalic monster with "Rhinydymie" (duplication of the nose and mouth), spina bifida and diaphragmatic defects associated with malposition of the viscera.

Anencephaly, a not infrequent malformation of the nervous system, is characterized by the absence of both cerebral hemispheres and usually a dark red mass of vascular tissue replacing the calvarium.

Scientific approach toward the problem of monsters began as early as the seventeenth century and became especially prominent in the eighteenth century.

In the early part of the nineteenth century Meckel suggested that monsters might be explained on an embryological basis. Then there followed a period during which pressure and other mechanical factors were looked upon as causative agents; in later years chemical factors were blamed. Later, von Recklinghausen attributed anencephaly to arrested closure of the primitive neural groove, a view generally accepted at present. Today the question may be raised whether teratogenesis can be explained on such environmental or hereditary bases. Jordan postulated that the development of monsters is the result of both heredity and environment and said, "... perfect development would require that both be perfect. Various degrees of imperfection or unfavorableness, in either or both, result in the endless degree of variations, anomalies, malformations, and monstrosities."

Nañagas, who studied 57 cases of anencephaly (43 females and 12 males, and 2 of an undetermined sex), found that in comparing body dimensions of normal with anencephalic fetuses the latter had a characteristic growth rate disturbance which resulted in body pro-

* Read before the Chicago Pathological Society, January 14, 1935.
Aided by a grant from the Rockefeller Foundation to the University of Chicago.
Received for publication April 8, 1935.

portions that were almost constant for all anencephalics. He concluded that some factor during intra-uterine life caused the body to assume abnormal proportions, but once these were assumed the body resumed its normal growth.

Experimental embryology has contributed evidence in regard to the factors in the production of monsters. Particularly significant are the experiments of Stockard, Child, and their students, on the effects of anesthetics on the developing embryo. Child's gradient hypothesis, originally introduced in 1915 and referring to the fact that organs which are developing most rapidly at the time of interference suffer most, is still valid.

Anomalies of the endocrine glands are frequently found in monsters but the exact relationship is not understood. Mattina reported 3 cases of anencephaly with changes in the endocrine glands and concluded that thyroid deficiency is in part responsible. Although normal suprarenals in monsters were reported by Barlow, Browne is of the opinion that the adrenals may be entirely absent in anencephaly. According to him the zona fasciculata, which in the full term infant represents only a small fraction of the total cortex, may be as broad in the anencephalic as in the adult. He concludes that the latter results are due either to a failure of development or to the destruction of the pituitary body.

Ettinger and Miller found adrenals absent in only 2 of their 9 cases. In the cases where they were present the disturbance was confined to the cortex, the medulla appearing normal microscopically. Only 8 of the 9 cases studied had an anterior lobe of the pituitary body, and of those only 3 showed a pars nervosa. Josephson and Waller reported similar findings.

Kohn found the pituitary present in all of 11 fetuses but in none was it normal. The pars anterior was invariably present, the pars intermedia absent and the pars posterior was found in only 3 cases. He concluded that the small adrenals were a direct result of the malformation of the hypophysis.

Kiyono in 1925 found the pituitary gland in only 7 of 11 fetuses, and of these only 3 had a pars posterior. The adrenals weighed under 1 gm.

In 1919 Hofstätter demonstrated a definite increase in the size of the adrenals after pituitary injections.

The incidence of monsters is not definitely known. According to

Bean, of every 100 pregnancies 80 end in normal births, 7 are aborted as pathological ova, 12 are aborted as embryos or fetuses showing various degrees of abnormality, and 1 pregnancy results in a monster. Tracy quotes Spangler, who studied 11,521 births and reported the incidence of anencephaly as 1 in every 900, or approximately 0.1 per cent.

Exact data on the frequency of anencephaly in families are not available. A few illustrative cases may be quoted from the literature. Jensen reported multiple births of monsters in a para IX. The first was a 5½ month fetus with spina bifida; then 3 normal pregnancies occurred, followed by a full term infant with spina bifida which lived only 82 days; then a normal pregnancy which was followed by a full term stillborn infant with spina bifida; the eighth was a full term child who had a meningocele and internal hydrocephalus, and the last was a stillborn anencephalus.

Thoms reported 3 anencephalic births in the case of one woman. Anencephaly is rare in twins. Thompson described a case of twins in which one was anencephalic. Still less frequent are conjoint anencephalic monsters. Mudaliar described an anencephalic thoracopagus dibrachius dipus. There is no mention in the literature, however, as far as I could ascertain, of anencephalic separate twins.

REPORT OF CASE

Clinical History: A negress, aged 30 years, para II, gravida III, had labor induced after a diagnosis of polyhydramnios had been made. Two days afterward the membranes ruptured spontaneously, and 5 hours later she was delivered of an anencephalic female monster. Five minutes after the birth a normal placenta was delivered. The membranes and cord were also normal.

The anencephalic monster, delivered by head presentation, weighed 5½ pounds. It began to breathe spontaneously; the pulse rate was 124 and the respiratory rate 30 per minute. One hour and eight minutes after delivery it died and the body was then placed in formaldehyde. Autopsy was not performed for 3 weeks.

AUTOPSY REPORT

The body was that of a full grown anencephalic monster delivered at term. The calvarium was missing and a hemorrhagic soft membrane, known as area cerebrovasculosa, was present, extending from a region just above the eyes to a level corresponding to the occipital protuberance. This meningocele measured 63 mm. transversely, 62 mm. midsagittally and 45 mm. from base to tip. The remainder

of the scalp was covered with hair. The right eye seemed to be well within its socket and measured 17 mm. in width, while the left eye had a narrow upper lid which was proptosed and measured 22 mm. in width (Fig. 1A). The proximal part of the bridge of the nose was missing, being replaced by a membrane which extended from the hemorrhagic membrane which replaced the calvarium. The right nostril was covered by a hump-like soft structure, while the left was almost flat. The width between the outer surfaces of the alae nasi was 25 mm., while that between the inner surfaces was 16 mm. The right nostril was oval, its diameter being one-third of that of the left, which was cleft-like and measured 7 mm. The region of the nasal septum was depressed and continuous with a groove running down a median elongation of the upper lip, which was fused with the lower lip on the left side only (Fig. 1A). It formed the median boundary of a blind oval pouch. Two apertures were thus encountered: on the right a triangular-shaped one (the true mouth) 13 mm. wide, leading into the oral cavity; and on the left a slit-like aperture which led into a blind pouch 12 mm. deep (the false mouth), which had no communication with the oral cavity and was only 3 mm. wide. Thus, there was a complete right and an incomplete left harelip. The ears were grossly normal.

The neck was absent. The chest and abdomen appeared normal anteriorly, but posteriorly (Fig. 1B) there was a depression in the region of the third lumbar vertebra about 28 by 23 mm. in diameter which was covered by a thin dark skin. In the center of the depression was protruding a round soft mass of tissue about 13 mm. in diameter. The external genitalia and extremities appeared normal.

After the soft spongy wall of the meningocele was reflected, fibrous tissue and blood clots were encountered. No trace of cerebrum or cerebellum could be made out. Of the cranial nerves, the right optic and both infra-orbitals were recognized; on microscopic examination I was able to make out the left optic and the seventh and eighth nerves. The medulla oblongata was intact.

A section from the meningocele showed it to be made up of large cells with foamy or vesicular cytoplasm and a small, round or oval eccentric nucleus. A section from the brain site consisted of hemorrhagic connective tissue in which blood vessels, smooth muscle and nerve tissue elements were easily discerned. It was lined with cuboidal epithelium over vascular papillary projections — all re-

sembling the choroid plexus. Nothing that could be identified as hypophysis was found.

The muscles of the right eye were degenerated and so distorted that none could be made out. The eyeball, when cut open, revealed practically no retinal tissue but only a lens with a strip of tissue attached to it. The left eyeball with its muscular attachments was poorly preserved and a section of it showed all eye structures to be well developed except for the iris and ciliary body. The retina was completely detached and thrown into folds — probably an artefact. The optic nerve was normal, though the demarcation between the nerve bundles and the interstitial septa was not plain. There were numerous hemorrhages within the eye tissues but these were most likely due to postmortem changes.

The internal acoustic meatus was 5 by 5 mm. on the left and 4 by 4 mm. on the right. The auricles, external canals, Eustachian tubes and antrums were normal. Microscopic examination of the right ear showed the cavum tympani to be apparently normal. The malleus, incus and one crus of the stapes were intact. The membrana tympani as well as the stapedius and tensor tympani muscles appeared normal. The inner ear showed the vestibule, saccule, utricle and the semicircular canals to be normal. The cochlea was egg-shaped and the modiolus rudimentary. The internal auditory meatus was larger than normal and in the region of the crista transversa there was a deficiency of bone. All the neuroepithelium revealed extensive postmortem changes, precluding a more exact diagnosis.

The left ear showed an apparently normal cavum tympani and drum membrane. The malleus and incus were present. The incostapedial joint was made out; the stapedial head, however, did not join the crura, nor was there a normal footplate. The posterior joint was present as well as the seventh nerve; the eighth, however, was greatly distorted. Both middle ear muscles were present. The stapedius muscle did not join the stapes. The inner ear showed, aside from the three canals, a peculiar short vertical canal anterior to the vestibule and above the level of the cochlea. Its connection could not be determined. The saccule and utricle could not be identified. The vestibule contained in part a very vascular nerve tissue. The round window appeared intact; the cochlea was oval in section and the modiolus was deficient. The internal auditory

meatus was very large and, as on the right side, the bone appeared deficient in the region of the crista transversa.

In brief, the abnormalities consisted of deformities of stapes, abnormal meatus and modiolus on both sides.

Figure 2 shows the two distinct nasal capsules separated by a median sulcus. The right capsule is cartilaginous and wider than the left, which alone is covered by a prolongation of the ossified maxilla. The imperfectly developed asymmetrical orbits appear in Figure 2. The relations of the Eustachian tube were normal, but there was no communication between the nasal cavities and the pharynx. The maxillary and ethmoid sinuses, as well as the larynx, epiglottis and tongue (Figs. 3A and 3B) were normal.

When the head was cut midsagittally it was found that the floor of the left nasal capsule was supported by a mass of tissue which formed the median wall of the "accessory mouth" which is about to be described ("a" in Figs. 3A and 3B). Figure 3A shows the wall intact, while in Figure 3B this space is occupied by the accessory mouth. The length of this mass of tissue, as measured in the center, was 20 mm. from the floor of the nose to the roof of the mouth, as compared to only 5 mm. on the right side. When this median wall was removed a space was encountered in which there was no pharynx or uvula but only a tongue-like bit of tissue 2 mm. long. Histologically it consisted of connective tissue covered with stratified epithelium, without glands or muscle.

The lining of the accessory left mouth had the typical appearance of an oral cavity. The lateral wall of this mouth had six tooth germs, which was also the case on the other side. There were the normal ten in the mandible. As mentioned before, the right mouth was wide with a correspondingly broad maxilla. The tongue was 37 mm. long and the base was 17 mm. thick.

The spinal cord from the medulla to the level of the third lumbar vertebra was normal. The vertebral arches of the third and the fourth lumbar vertebrae were missing; the cord was protruding and came to the surface under the thin membrane (Fig. 1B).

The thymus measured 55 by 30 by 14 mm. In spite of extensive postmortem changes the lobular character of the gland could easily be discerned, as well as the medulla and the cortex with its Hassal's corpuscles.

The thyroid gland, the lower part of the trachea and the main bronchi appeared normal.

The left lung showed a partial fusion of its upper and middle lobes, and the upper lobe revealed superficial markings suggestive of a division to form a third lobe. The right lung had four lobes, the upper lobe being divided by a deep groove running anteroposteriorly to form the fourth. The sectioned lung, aside from great distention of the alveolar spaces, showed no abnormality.

The pericardial cavity, the heart and the great vessels were normal. The abdominal cavity was separated from the thoracic by an abnormal diaphragm whose two halves were not in the same plane. The right half of the diaphragm was lower than the left and the two halves were connected posteriorly by a delicate membrane which formed a pouch extending from the left side into the thoracic cavity on the right, above the right half of the diaphragm and in contact with the posterior surface of the right lung. The upper pole of the spleen, which occupied this pouch, was afforded therefore an intrathoracic position.

The esophagus lay slightly to the right of the midline and was dilated into an imperfectly developed stomach which was partially thoracic in location and lay somewhat to the right of the midline. The "stomach" extended downward in the longitudinal axis of the body, passed anteriorly to the spleen and curved slightly to the left lobe of the liver, where it ended abruptly in a sphincter-like ridge which separated it from the thin-walled duodenum. The latter extended distally almost to the midline, curved slightly to the left and ended in a hardly perceptible duodenojejunal angle. Both the large and small intestines were attached posteriorly by a common mesentery.

The liver and pancreas were normal in gross. Microscopically the architecture of the organs could not be made out because of extensive autolysis.

The adrenals could not be identified in gross or recognized microscopically.

The left kidney consisted of a spongy mass measuring 40 by 16 by 12 mm. The right kidney was 40 by 19 by 16 mm. and like the left appeared honey-combed. Microscopically cellular structure could not be made out because of postmortem changes. Only a bare outline of the cortical and medullary structure could be detected.

In gross the Fallopian tubes and ovaries appeared normal. In sections of the latter there were no Graafian but only primordial follicles.

DISCUSSION

The case reported may be interpreted as one indicating a tendency toward duplication of facial structures. The following series may be recognized.

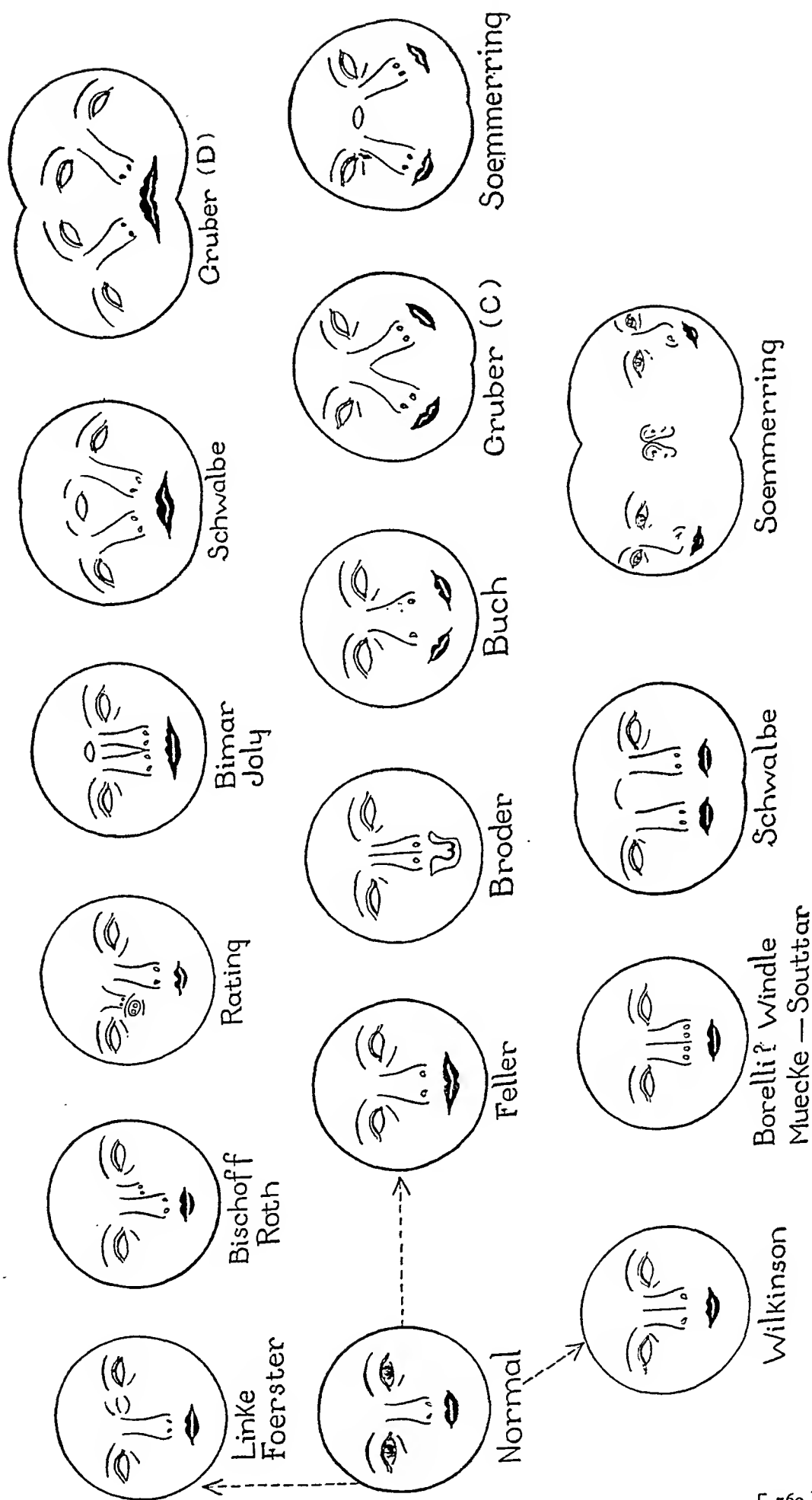
The case of Feller (Text-Fig. 1) showed doubling of the lower jaw with partial doubling of the tongue. My case showed doubling of the lower jaw with partial doubling of the mouth. In Buch's case there was one broad nose and within it a rudimentary second nose; there were two oral cavities and only two eyes, in fact the upper third of the face was normal.

The case of Gruber (Text-Fig. 1) showed two distinct and separate noses and mouths. Soemmerring's case presented a doubling of the oral cavities and nose and the appearance of a third eye.

Förster (Text-Fig. 1) reported a case of doubling of the left side of the frontal bone with the formation of two eye orbits, eyelashes and eyebrows but without a simultaneous doubling of the eyeball. In this mulatto male, 8 months old, who was normal in every respect except for the anomalies of the cranium, there was a small eye cleft with lids and eyebrows but no eyeball on the left side in the usual place, and then somewhat laterad a third larger cleft in which there were a ball, eyelids and eyebrows. On the left side there was a hydrocele. The nose was flat and had only one ala nasi and a blind nostril on the right. The right eye and mouth were normal in gross.

Doubling of the nose is not infrequent. One is dealing here with a broadening of the medially situated nose which, because of an invagination of its medial parts, has led to the formation of a nose called "Doggennase" by Bumba and Lucksch (Text-Fig. 1). The "Doggennase" (congenital malformation in which the nares are divided by a groove) is a result of the failure of the two overlying bones which develop lateral to the cartilaginous nasal septum to unite. As a result of this the septum becomes wider and the nostrils farther apart. Sinking of the septum or its atrophy results in the formation of a double nose which has only one nostril on each side, as in my case.

Rating reported a case (Text-Fig. 1) in which there were something resembling a nose in the region of the right eye, harelip, cleft palate and spina bifida along the entire spinal column. There was a somewhat flat "main" nose in the midline, the right nostril of which



TEXT-FIG. 1. — Modified after a schematic drawing by Bernard Rating in *Virchows Arch. f. path. Anat.*, 1933, 288, 236.

was contracted and the left wide. The accessory nose lay between the root of the nose and the right eye and seemed to have sprung from the orbital roof. Underneath this accessory nose there was, on the right side, a fissure 10 mm. long which was covered with cilia. This contained an eyeball and two lenses, an optic nerve of its own but no cornea; according to the author this was no doubt an anlage for a cyclopic eye. There was, however, no nasolachrymal duct to the accessory nose, which covered the right eye from above. The left eye, in which no tear canals could be made out, was somewhat larger than the right eye, which was 27 mm. from the midline, while the left eye was only 10 mm. from it.

According to Hübner, "all gradations of a diprosopia ranging from a doubled hypophysis through a 'Rhinodymie' and ending with a diprosopus sensu strictiori are possible." In the latter case the skull is incomplete, the face incompletely or completely doubled and this doubling may range from "Rhinodymie" to di-cephalus.

Lasagna reported a case of double nose in a child, 2 months old, with no other abnormalities. The left nose had a small aperture, while the right was normal in every way. The pinhead-sized opening referred to and interpreted by the author as a rudimentary "tear collector" was in the left infra-orbital region.

Bischoff and Roth (Text-Fig. 1) have described a case in which one nose was in the normal midline position while the other had its origin from above the left orbit. There were four frontal bones and the mouth was normal.

The case of Bimar, a typical "Rhinodymie," was first recognized in 1881, and since then some 6 cases have been described.

A further step toward duplication of the face is represented by the case of Schwalbe in which there were two mouths, two noses and three eyes (a true "Rhinodymie"). Finally, the last stage is exemplified by Gruber's case (last drawing in the first row) in which there were four eyes, two noses and two mouths.

Wilkinson (Text-Fig. 1) described a deep depression in the midline of the nose, wide separation of the nostrils, and broadening of the whole feature. On the inner side of each vestibule the anterior ends of the nasal septum could be seen as a proximal ridge. The two sides of the septum having been separated from one another, there was no nasal obstruction. He pointed out that failure of the median

walls of the nasal cartilage to fuse is rare, and that such a failure might account for a median cleft palate and a bifid nose.

A further stage toward duplication is presented in the cases of Windle, and Muecke and Souttar. Windle (Text-Fig. 1) reported the case of a girl, aged 5 years, who had a nose that was incompletely divided into two parts by a longitudinal furrow. There were four nostrils, two on each side of the septum, and two more laterad. The two median ones were functionless, small, blind and only 10 mm. deep. The normal, wider pair functioned properly. The only other malformations were two ridges on the upper lip. The author believed that this represented an attempt at division of the nose into two noses.

Muecke and Souttar, who reported a case of two completely formed noses in a girl, aged 3 years, the right being more central and larger than the left, but each having its own septum, nostrils and well formed alae nasi, postulated that the nature of such an anomaly depends on whether or not the two anlage are present. When they are present the medial depression is not flat but is deeply grooved, so that two equally well developed noses lie one next to the other, each with its individual septum, alae and nostrils. Finally, there are stages of symmetrical doubling represented by the cases of Schwalbe, and especially that of Soemmerring (Text-Fig. 1).

Thus, there may occur all gradations of doubling from incomplete to that of diprosop, tetraphthalmic, tetrorbitus, diotus, monosomus, dignathus and monauchenos.

In summary, it may be said that monsters of this type may be divided into three series, as shown in Text-Figure 1. The tendency in the first row is toward duplication of nose and eyes, eventually reaching the mouth. In the second row the mouth and nose are involved at first with a tendency to include the eyes. In the third row all the elements are duplicated.

SUMMARY AND CONCLUSIONS

1. The case reported belongs in the group of "Rhinodymie," as described by Rating, and forms a link between the case of Feller, showing doubling of the lower jaw only, and that of Buch with two well developed and distinct mouths and only a rudimentary second nose (Text-Fig. 1).

2. The anatomical findings and the consideration of cases described in the literature suggest that doubling of the nose and mouth are expressions of a tendency toward dicephaly. These were associated in the case reported with anencephaly, deformities of stapes, meatus and modiolus on both sides, defect of the diaphragm, imperfectly developed stomach, absent adrenals and spina bifida.

3. Experimental evidence, which alone is significant, indicates that anencephaly is due to an inhibition in the early development of the embryo affecting regions of highest rate of development. The frequent association of anencephaly with cephalic duplications suggests that the latter may be due to similar factors.

4. The deficient diaphragm may be interpreted as an interference with the development of the pleuroperitoneal membrane.

5. Much of the speculation concerning the causes underlying human monsters is futile. The only direct evidence bearing on the subject at the present time is that of experimental embryology.

NOTE: This paper was inspired by Dr. George W. Bartelmez, Professor of Anatomy, University of Chicago. I also wish to acknowledge my indebtedness to Dr. Robert S. Jason, Assistant Professor of Pathology, Howard University School of Medicine, for the study of microscopic sections of the viscera; Dr. Elmer W. Hagens, Assistant Clinical Professor of Laryngology and Otolaryngology, Rush Medical College, for the study of the sections of the ears; and to Dr. Max L. Folk, Assistant Professor of Ophthalmology, College of Medicine, University of Illinois, for the study of the eye sections.

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DESCRIPTION OF PLATES

PLATE 110

FIG. 1. Anencephalic fetus.

A = ventral view.

B = dorsal view.



PLATE III

FIG. 2. View from above of the inner base of skull.

- a = free margin of squama occipitalis.
- b = free margin of temporal bone.
- c = internal acoustic meatus.
- d = right cartilaginous nasal capsule.
- e = maxillary region of skull.
- f = left eyeball.
- g = right optic foramen.
- h = medulla oblongata in foramen magnum.

FIG. 3A. Two halves of the head separated to show the accessory mouth.

- a = median wall of the accessory mouth underneath the left maxilla.
- b = base of tongue.
- c = left turbinates.

FIG. 3B. View from above of the medial side of the left moiety.

- a = space occupied by the accessory mouth.
- b = tongue in true mouth.
- c = epiglottis.
- d = left turbinates.
- e = ossified centrum of the third cervical vertebrae.



ANOMALIES OF THE CIRCLE OF WILLIS WITH RESULTING ENCEPHALOMALACIA AND CEREBRAL HEMORRHAGE *

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INTRODUCTION

There is an increasing tendency today to attribute to functional abnormalities the anatomical changes that are undoubtedly the result of vascular lesions. This pertains particularly to encephalomalacia and cerebral hemorrhage. Fischer-Wasels¹ stated very recently that in instances of brain hemorrhage there is primarily a destruction of brain tissue as a result either of trauma or of toxins, or because of local circulatory disturbances. As a result of the primary destruction split products may be present which secondarily damage the walls of blood vessels, either by accentuating the local disturbances of circulation or by producing anatomical changes in the vessels which finally may result in necrosis. The damage to the walls of the vessels may also cause reflex disturbances. Weil,² realizing the difficulty in evaluating the different theories of explanation of cerebral hemorrhages and encephalomalacia, stated that different possibilities may exist, primary vascular disease or primary functional disturbances of vasomotor regulation. Although it cannot be denied that apparent vascular lesions may very occasionally be the result of functional disorders which cannot be demonstrated by means available to the morphologist, it cannot be emphasized sufficiently that every possible morphologically demonstrable cause of vascular lesions must be searched for, carefully evaluated and ruled out before the morphologist resorts to an explanation based on functional disorders. The assumption of functional disorders is often a confession by the morphologist of inability to find the real cause of the lesion in question.

In the following, 2 cases of encephalomalacia and cerebral hemorrhage will be reported. In both of these, vascular disturbances were not the result of an occlusion of the vessels, and the causes of these

* Received for publication March 28, 1935.

lesions were at first difficult to determine. Only a careful examination of the vessels revealed anomalies of the circle of Willis as the underlying cause of the brain abnormalities. Also, a third case of anomalies of the circle of Willis not accompanied by vascular disturbances of the brain will be reported.

The literature on this subject is very scant. It may be divided into three parts — one dealing with the onto- and phylogenetic development, one statistical, and one associating the anomalies with certain psychic features. It may be of interest to note that anatomical brain lesions, as far as could be ascertained, were not correlated with anomalies of the circle of Willis. There are, however, references to cerebral aneurysms associated with anomalies of the arteries of the circle of Willis, and the opinion is expressed that abnormalities in the distribution of the blood because of such anomalies may be a mechanical factor in the causation of cerebral aneurysms (Jacques³). Only the more important references pertinent to this communication and principally confined to the posterior communicating branches of the circle of Willis are given.

LITERATURE *

Webber⁵ in 1882 described a case of an abnormal distribution of the circle of Willis. The right posterior communicating artery was one-tenth its usual diameter and the right posterior cerebral was twice its usual size. The left posterior communicating artery was nearly twice its normal size and was virtually the origin of the left posterior cerebral artery.

Windle⁶ in 1888 examined 200 brains and found both posterior communicating arteries extremely small in seven instances. Both were absent in three. He stated that the origin of the posterior cerebral artery from one or both internal carotid arteries was the most common variety.

De Vriese⁷ in 1905 stated that in fish, amphibians, reptiles, birds and some mammals the internal carotid artery provides the only arterial supply to the cerebrum. Upon entering the cranial cavity each internal carotid divides into two branches, the cranial and the caudal. The terminal branches of the caudals unite to form the

* The earlier literature is given in Mitchell's⁴ article.

basilar artery. In mammals the circle of Willis is formed either by branches of the internal carotid arteries (so-called "primitive type") or is formed by branches of the vertebral arteries (so-called "recent type"). The following anomalies of the posterior communicating arteries were found.

- (1) The caliber of these vessels may be larger than normal.
- (2) Variations in the caliber of the right and left arteries.
- (3) Complete absence of one or both vessels.
- (4) One or both vessels may be so large that the posterior cerebral artery appears to be a terminal branch of the posterior communicating. The divisional branch of the basilar artery up to the point of union with the posterior communicating branch (most proximal portion of the posterior cerebral) may be so small that it appears to be the posterior communicating artery.
- (5) The basilar artery may be formed by the fusion of the posterior communicating and the divisional branch of the basilar artery. (The posterior communicating artery and the divisional branch of the basilar artery are equal in size.)

Blackburn⁸ in 1907 stated that an enlargement of one or both posterior communicating arteries was a common anomaly. In almost every specimen enlargement of the posterior communicating arteries coincided with small posterior trunks at their point of origin from the basilar or at their proximal portions.

Stopford⁹ stated in 1916 that in 105 brains out of 111 there was a complete anastomosis of the circle of Willis. In 6 of the remaining brains the circle of Willis was incomplete because of the absence of the posterior communicating branch on one or both sides.

Berger¹⁰ in 1923 described the transformation of the left posterior communicating artery into a fibrous cord. The right posterior communicating artery was very large. The right posterior cerebral artery seemed to be a continuation of the right posterior communicating artery.

Walcker¹¹ in 1924 stated that there are two types of circle of Willis. One is characterized by its patency, the other by an interruption of its continuity. The former is referred to as "closed" circle, the latter as "open" circle. The closed circle of Willis is found frequently in individuals with a mesocephalic and a slightly brachycephalic configuration of the head. In the decidedly brachycephalic there often is an increased number of anastomoses seen in

the circle of Willis. The open circle of Willis is often present in individuals with dolichocephalic configuration of the head.

Shellshear¹² in 1926 described the arteries of the brain of an orangutan and stated that the arteries of the brain are stable phylogenetically and ontogenetically.

Jacques³ in 1926 found a circle of Willis abnormally incomplete. The right posterior communicating artery took the course normally taken by the right posterior cerebral artery. At the site of origin of the left posterior communicating artery was an aneurysm measuring 2.5 cm. in greatest diameter.

Voris¹³ in 1928 described the arterial supply of the brain in the Virginian opossum (*Didelphis virginiana*) and found a double communication between the internal carotid and the basilar artery. One was formed by the posterior communicating and the second one by a small branch of the medial cerebral artery which came off immediately after its origin. There was no anterior communicating artery.

Hindze and Fedotowa¹⁴ in 1931 described a case of absence of the posterior communicating artery on one side. This vessel on the other side was very large and seemed to be the origin of the posterior cerebral artery. These authors described seven types of anomalies as follows:

(1) *Primitive Type*: The posterior cerebral artery forms the continuation of the internal carotid artery. The posterior communicating artery is larger than normal.

(2) *Transitional Type*: The posterior cerebral arteries are formed about evenly by the posterior communicating and the divisional branches of the basilar artery.

(3) *Recent Type*: The posterior cerebral artery is formed principally by the branches of the basilar artery.

(4) The internal carotid and basilar arteries are united by an embryonal trigeminal artery, or (5) hypoglossal artery.

(6) *Mixed Type*: On one side the posterior communicating artery is absent and on the other side it is much larger than normal.

(7) Complete separation of the circulation of the internal carotid and vertebral arteries.

De Vriese⁷ found anomalies of the circle of Willis for the most part in criminals and in the insane.

Blackburn⁸ pointed out that in only 65 out of 220 brains of consecutive cases of mental disease was the circle of Willis normal.

Hindze¹⁵ found anomalies of the circle of Willis in a schizophrenic, in a criminal, and also in a number of outstanding individuals — scientists and poets.

Wyrubow¹⁶ stated that anomalies of the anterior cerebral arteries were found in 22.3 per cent of the insane and in criminals.

Parnizetti¹⁷ found anomalies of the circle of Willis in 55.1 per cent of criminals examined.

CASE REPORTS

CASE I. *Clinical History:* A 50 year old female was treated intermittently with iodine over a period of 6 years for a toxic goiter. Her complaint on admission to the hospital was shortness of breath, palpitation and swelling of the legs. Physical examination revealed an undernourished patient. The heart was enlarged and there was a systolic murmur at the apex. The liver and spleen also were enlarged. She had an anxious expression and a stare, but no exophthalmus. The right lobe of the thyroid was enlarged. The basal metabolic rate was + 79. She was given iodine and her basal metabolic rate dropped to + 49 in 3 days. Digitalis was administered and surgical removal of the thyroid was contemplated but not performed. Seventeen days after admission her temperature rose to 103.6° F. The white count at that time was 6900. The temperature dropped to normal but occasionally rose again. She developed marked psychic symptoms such as incoherent talking and crying, and became irrational. Later the temperature rose to 106.4° F. and she died of what was considered to be thyroid crisis.

Autopsy Report

At autopsy there was a recent and organizing bronchopneumonia and a fatty infiltration of the heart involving mainly the right ventricle. There was a coronary arteriosclerosis and myocardial fibrosis, dilatation of the heart, chronic passive hyperemia of lungs, liver, spleen and kidneys and edema of the lower extremities. A nodular colloid goiter was found which histologically revealed foci of hypertrophy and hyperplasia. The vessels of the base of the brain showed the following abnormalities. The left posterior cerebral artery took its origin from the left internal carotid artery by means of a large posterior communicating artery. There was a fibrous cord without any recognizable lumen running between the first portion of the left posterior cerebral (or proximal portion of the posterior communicating) and the end portion of the basilar artery where the posterior cerebral artery starts normally. The right posterior cerebral artery

took its origin from the basilar artery. There was no communication between the right internal carotid and the right posterior cerebral artery. The anterior cerebral artery and the anterior communicating branch were normal. A moderate degree of arteriosclerotic change was found throughout the arteries of the base of the brain. Multiple sections of the brain revealed two areas of encephalomalacia which were rather recent and which were found in the white substance of the cerebrum close to the right lenticulate nucleus. Small areas of hemorrhage were also found in the vicinity of the encephalomalacia.

Histological examination of the brain revealed, in addition to encephalomalacia and hemorrhage, various degenerative changes of the ganglion cells. Many of the nuclei were absent, the cytoplasm of these cells was not well defined, was pale, and in many fields only shadows of ganglion cells could be made out.

Summary

A 50 year old female developed mental symptoms, became irrational and died of what was considered to be a thyroid crisis. At autopsy, in addition to other pathological findings, encephalomalacia and cerebral hemorrhages were found. There was a complete interruption of the circle of Willis and no communication between the internal carotid and the vertebral arteries. There was an arteriosclerosis of the vessels of the base of the brain.

CASE 2. Clinical History: This patient was an adult white male, 70 years of age, whose blood pressure had been elevated for a number of years. For the past 10 years he had experienced attacks of complete paralysis affecting the extremities and face, with loss of consciousness. These attacks lasted about 3 or 4 days; occasionally, however, only a few moments. More commonly the left side of the body was involved. Four months before death he had an attack of precordial pain which was interpreted as coronary thrombosis. His mental condition most of the time was good, although in the last few months preceding death there had been periods of mental disturbance. The arterial blood pressure was constantly around 220/150. During the last few weeks of life he had hematuria. Bronchopneumonia developed and he died shortly afterward.

Autopsy Report

At autopsy there was a generalized arteriosclerosis with marked involvement of the coronary arteries and occlusion of several branches. There were old and recent infarcts in the myocardium of

the left ventricle, dilatation of the heart, chronic passive hyperemia of lungs, liver, spleen and kidneys, and edema of the lower extremities. A nephrosclerosis of the arteriolar variety, hypertrophy of the heart and a bilateral confluent bronchopneumonia were also found. There was a carcinoma of the urinary bladder. The arteries of the base of the brain revealed the following abnormalities: The right posterior communicating branch of the circle of Willis was absent and the left posterior communicating branch was transformed into a very thin fibrous cord. In the anterior-superior portion of the left frontal lobe near the longitudinal fissure there was an area of hemorrhage measuring 0.5 cm. in diameter. This lesion was located partially in the gray and partially in the white matter. An area of softening was observed anteriorly to the anterior horn of the left ventricle close to its wall. An area of old encephalomalacia was seen in the right anterior portion of the corpus callosum. The surrounding white substance showed small, yellowish-tinged cystic cavities. Close to the left internal capsule another area of encephalomalacia was found which measured 1 cm. in diameter. The left parietal lobe revealed a deep red area of hemorrhage within the white matter extending over an area 0.5 cm. in diameter. Frontal sections through the right occipital lobe revealed a soft, yellow, necrotic area which measured 1 by 2 cm. in diameter. A small red area of hemorrhage was also found in the gray matter of the midportion of the occipital lobe.

Histological examination of the brain revealed areas of recent and old encephalomalacia and hemorrhage. Small cysts were seen which were lined with phagocytic cells containing many yellowish brown pigment granules. Other fields showed many scavenger cells and only indistinct outlines of brain tissue. The cytoplasm of many ganglion cells was pale and often vacuolated. The glia nuclei were preserved.

Summary

The brain of a 70 year old man whose main clinical symptoms were attacks of unconsciousness revealed an interruption of the circle of Willis and resulting separation of the circulation of the internal carotid and vertebral arteries. There was a marked arteriosclerosis of the arteries at the base of the brain and multiple areas of encephalomalacia and hemorrhage into the brain.

CASE 3. *Clinical History:* A 65 year old male complained of rapidly increasing shortness of breath, nocturnal cough, and swelling of the ankles. On physical examination marked cyanosis was present and the cardiac dullness was enlarged. There was a loud systolic murmur over the entire precordium. The patient was mentally confused during the last 10 days of his life. The clinical impression was a generalized arteriosclerosis with coronary arteriosclerosis and myocardial fibrosis.

Autopsy Report

The autopsy revealed a marked generalized arteriosclerosis with involvement of the aortic valve and stenosis of its orifice. There was a coronary arteriosclerosis, myocardial fibrosis, hypertrophy and dilatation of the heart and generalized chronic passive hyperemia. There also was a bilateral bronchopneumonia. The circle of Willis showed the following changes: Both posterior communicating branches were much thinner than normal. Their lumens were not recognized. No openings were found at the usual sites of origin of both internal carotid and both posterior cerebral arteries. Multiple sections of the brain showed no gross changes.

The histological examination revealed marked degenerative changes of ganglion cells with necrosis in some instances. There were no areas of encephalomalacia or hemorrhages.

Summary

The brain of a 65 year old man who was mentally confused during the last 10 days of his life and who died of myocardial failure complicated by bronchopneumonia revealed a marked hypoplasia of both posterior communicating arteries. These vessels were reduced to the extent of prohibiting circulation of blood through them.

DISCUSSION

The first two brains are interesting in that both showed areas of encephalomalacia and cerebral hemorrhages in the absence of occluding lesions of the cerebral arteries. In each case there was a severe arteriosclerosis of the arteries of the brain and in each anomalies of the circle of Willis which principally involved the posterior communicating arteries could be demonstrated.

Stopford⁹ stressed the point that the posterior communicating arteries were of much greater importance during the early weeks of intra-uterine life than in the later periods. This is so because in the

early period they form the origin of the posterior cerebral artery from the internal carotid arteries. Later, when the posterior cerebral artery is reinforced by anastomosis with the basilar artery, the posterior communicating artery is no longer essential for the maintenance of the blood supply. Yet under normal conditions the channel between the internal carotid and basilar arteries remains open and can be used as a collateral vessel for either the internal carotid or basilar arteries and their branches. How frequently this channel is used in this compensatory fashion, and its importance, cannot be estimated. As the literature shows, and as was brought out by the 3 cases, the posterior communicating branches are not essential in the maintenance of the circulation of the brain under normal conditions. Since particular attention was paid to the posterior communicating arteries, changes in the caliber of these vessels were found quite frequently. Most commonly they were very thin. In instances of diffuse arteriosclerosis of the vessels of the base of the brain, however, the free unhampered collateral anastomosis of the circulation of the internal carotid and vertebral arteries seems essential, particularly in view of the fact that the cerebral arteries are end-arteries. In other words, an interference with the passage of blood through the circle of Willis does not make itself manifest until the circulation through the internal carotid and vertebral arteries is impaired. In the first 2 cases two causes for such impaired circulation are demonstrable, namely the arteriosclerotic plaques throughout the arteries of the brain, and the failing heart, evidence of which could be deduced from the findings of myocardial fibrosis, chronic passive hyperemia of the various organs, and edema of the lower extremities and the dilated heart. As a result of the impaired *vis a tergo* and of the arteriosclerosis of the vessels of the base of the brain, complicated by the complete separation of the two arterial channels of the brain, encephalomalacia and hemorrhages into the brain ensued. This conception of the cause of the brain lesion is based entirely on morphologically demonstrable lesions, not only of the brain itself but also of the various organs, particularly the heart. It may be mentioned in this connection that Fleming and Naffziger¹⁸ called attention to the danger of vascular disturbances of the brain in instances of a fall of the arterial pressure.

As was previously emphasized, the anastomosis between one posterior cerebral artery and the basilar artery was insufficient in the

first case. Stopford⁹ pointed out that under these circumstances, as was also seen in this brain, the posterior cerebral artery appears to be a branch of the internal carotid artery. He mentioned that what appears to be a compensatory enlargement of the posterior communicating artery to accommodate for its abnormally small origin from the basilar artery is strictly speaking a persistence of the embryonic condition.

Very interesting is Case 2. Clinically the many attacks of unconsciousness followed by paralysis of the extremities could not definitely be explained. At autopsy a number of areas of encephalomalacia and hemorrhage were found. Whereas it would have been difficult to explain the multiplicity of these lesions on anatomical grounds in the absence of multiple emboli or thrombi, the anomaly of the circle of Willis combined with the arteriosclerosis and anatomical evidence of failing heart, makes an explanation on morphological grounds possible. Of course it is impossible to state whether or not in addition to morphologically demonstrable lesions, physiopathological, merely functional conditions played an additional rôle in the causation of the infarcts. But, as a matter of principle, such functional causes should be taken into consideration only when a careful examination fails to yield an explanation based on morphological grounds.

In Case 3 the vessel anomaly and degenerative changes in the brain were the only significant findings. It is conceivable that the degenerative changes were the result of beginning deprivation of arterial blood supply. Similar degenerative lesions were also noted in the first 2 cases. It is of interest that all three patients were mentally confused during the last week of life. This confusion may have coincided with the beginning of the final myocardial failure.

As stated in the literature, anomalies of the circle of Willis have been correlated with psychic disturbances, since these anomalies have been found in a number of insane individuals and in criminals. On the other hand, such changes were also present in the mentally alert (Hindze¹⁵). The three patients who were the subject of this communication were mentally normal as far as could be determined by the investigation of the clinical records and by subsequent inquiries among relatives.

SUMMARY AND CONCLUSIONS

Anomalies of the circle of Willis, with resulting interruption of the circulation between the internal carotid and vertebral arteries, may form the anatomical basis of cerebral vascular disturbances. The recognition of such anomalies is significant because they aid in the explanation of cerebral hemorrhage and encephalomalacia on morphologically demonstrable grounds in the absence of occluding lesions of the supplying arteries.

In addition to local causes for encephalomalacia and cerebral hemorrhage one must consider also the condition of the myocardium and evidence of myocardial failure in the various organs.

Three brains are described which revealed anomalies of the circle of Willis involving the posterior communicating arteries, and an abnormal origin of the posterior cerebral artery in 1 case. Two of these brains revealed areas of encephalomalacia and cerebral hemorrhage, without the presence of occluding lesions in the supplying arteries. Whereas the posterior communicating arteries are not essential in the maintenance of the circulation of the brain under normal conditions, a free unhampered collateral anastomosis between the internal carotid and vertebral arteries is important in instances of diffuse arteriosclerosis of the arteries of the base of the brain combined with beginning myocardial failure.

This conception of the origin of these brain lesions is based entirely on morphologically demonstrable changes and does not require the assumption of theoretical functional disturbances of the circulation. Perhaps similar anatomical findings may explain anatomical changes elsewhere which now are attributed to functional disturbances.

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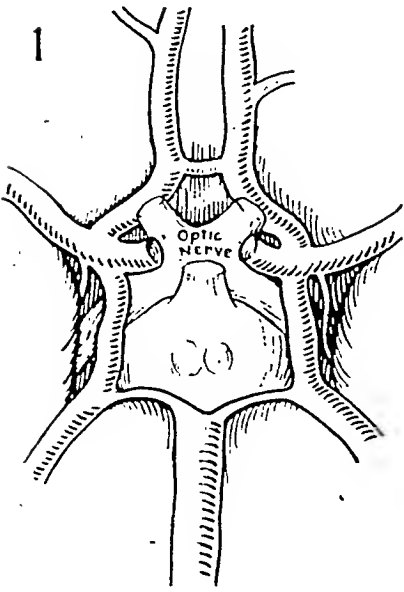
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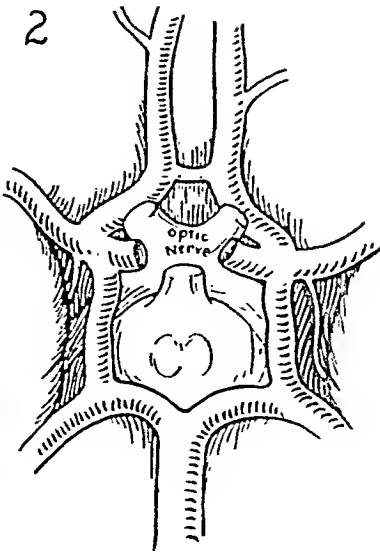
DESCRIPTION OF PLATE

PLATE II 2

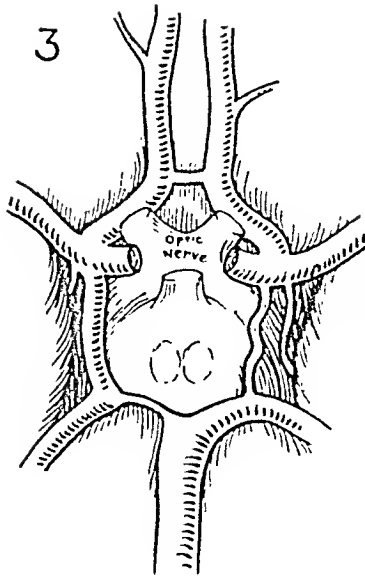
- FIG. 1. So-called *primitive type* of the circle of Willis. The posterior cerebral artery forms the continuation of the internal carotid artery. The posterior communicating artery is larger than normal. The branches of the divisional basilar artery are small.
- FIG. 2. So-called *transitional type* of the circle of Willis. The posterior cerebral arteries are formed by the posterior communicating and the divisional branches of the basilar artery. These two branches are of about equal size.
- FIG. 3. So-called *mixed type* of the circle of Willis. The left posterior communicating branch is normal (*recent type*) and the right posterior communicating artery corresponds to the *primitive type*.
- FIG. 4. The normal circle of Willis and the anomalies found in the first case are given for comparison. The left posterior cerebral artery takes its origin from the left internal carotid by means of a large proximal portion of the posterior communicating artery. The distal portion of the communicating branch and the divisional branch of the left basilar artery are transformed into a fibrous cord. The right posterior communicating artery is absent.
- FIG. 5. Case 2. The left posterior communicating artery is transformed into a fibrous cord. The right is absent.
- FIG. 6. Case 3. Both posterior communicating arteries are transformed into a fibrous cord.



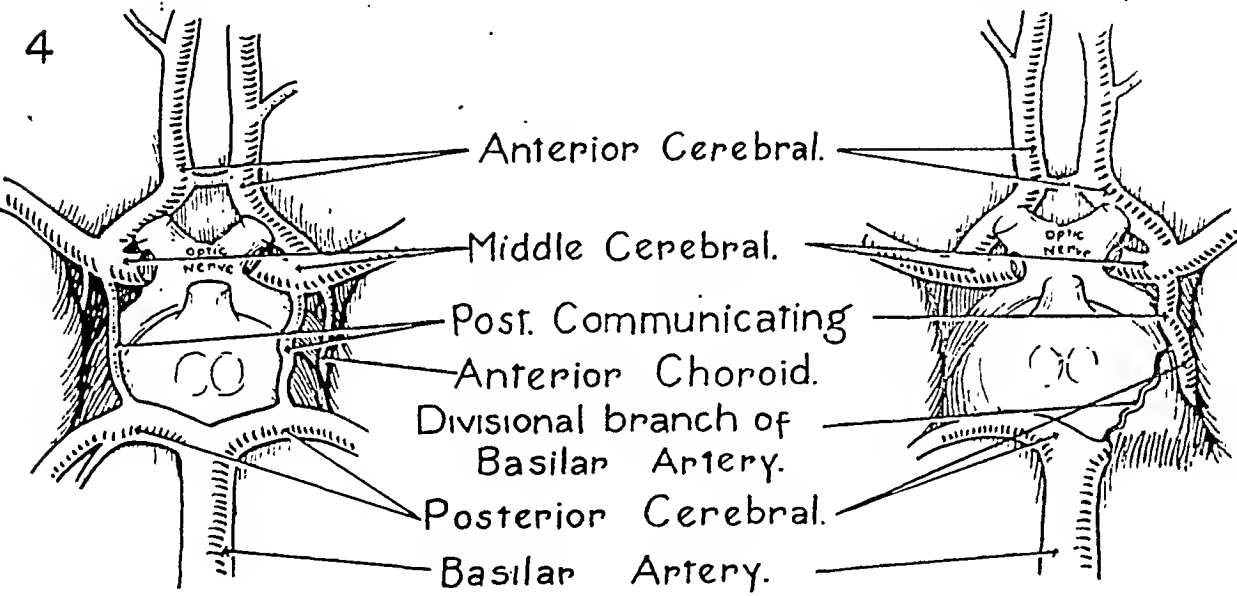
Primitive Type



Transitional Type.

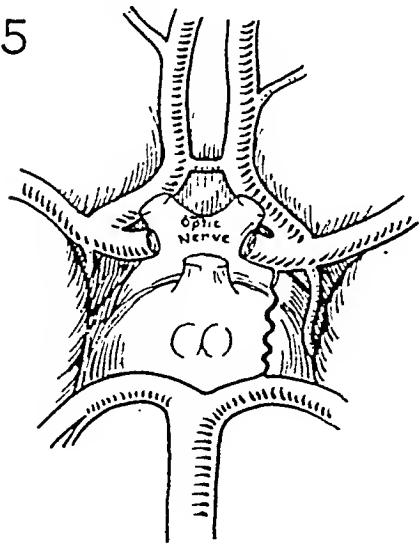


Mixed Type

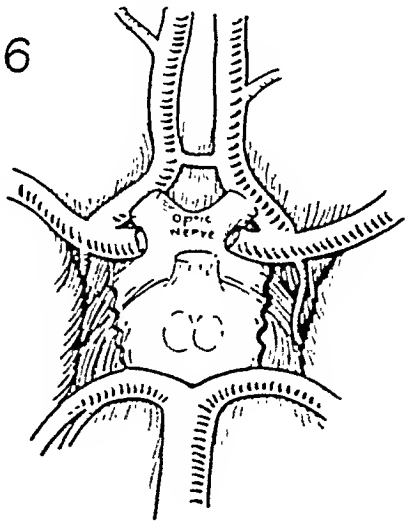


Normal

Case 1.



Case 2.



Case 3

EXPERIMENTAL GASTRIC EROSIONS FOLLOWING HYPOTHALAMIC LESIONS IN MONKEYS *

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INTRODUCTION

Cushing,¹ in his Balfour Lecture, has directed attention to some neurogenic factors involved in acute ulceration of the gastro-intestinal tract. In a comprehensive review of the literature on the subject he points out that the weight of evidence indicates a causal relation between hypothalamic disturbance and certain gastro-intestinal lesions.

Although it had long been suspected that the central nervous system plays a part in the genesis of gastro-intestinal ulceration (Schiff,² and Brown-Séquard³), the experiments of two Russian investigators, Burdenko and Mogilnitzki,⁴ were among the first to provide direct evidence that hypothalamic injury might lead to haemorrhagic ulceration in the stomach and duodenum. Using a subtemporal approach they produced small lesions in the base of the brain immediately behind the infundibular stalk. Gastric haemorrhages and erosions, acute ulceration and occasionally perforation with peritonitis resulted and in some instances, in animals surviving the operation by several months, chronic cicatricial ulceration was found. They explained their results on the basis of a destruction of a vasomotor centre in the posterior hypothalamus and of a metabolic centre in the tuberal region.

Keller, Hare and d'Amour,⁵ in a long series of experiments on cats and dogs in which many and varied types of experimental lesions had been made in the upper brain stem, found that apart from 3 chronic midbrain animals in which erosions occurred in the stomach acute gastro-intestinal changes most commonly followed lesions that had been associated with haemorrhage into the cerebral ventricles, *i.e.* after a transverse section of the brain at the level of the chiasma.

* Received for publication March 26, 1935.

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Multiple erosions of the stomach were encountered in some of the cases after hypothalamic injury. They occurred most frequently in the body of the stomach, rarely in the pylorus, and were most numerous on the crests of the folds.

Watts and Fulton⁶ have recently reported a series of experiments in monkeys in which localised hypothalamic injury was associated with acute gastro-intestinal changes. Of 17 animals with large hypothalamic lesions 4 developed gastric erosions, 1 died of a perforated duodenal ulcer and several showed mucosal haemorrhage. A careful examination of the gastro-intestinal tract in a control series of 63 animals revealed that gastric erosions were found in only 1 monkey without a hypothalamic injury, and this animal had received daily injections of ephedrin after a midthoracic transection of the spinal cord. Their observations did not permit any conclusions as to the relation between the destruction of any single group of hypothalamic nuclei and the pathological changes in the gastro-intestinal tract. They were inclined to the view that the erosions following lesions in the tuberal and supra-optic regions were due primarily to local ischaemia incident to hyperactivity of the sympathetic vasoconstrictor mechanism of the gut.

As the observations of Watts and Fulton⁶ were based on relatively long term experiments, they suggested that further studies were needed with earlier sacrifice; the present experiments have been undertaken with this object specifically in view.

METHOD

This report is based on a study of 19 monkeys (11 capuchins, *Cebus fatuellus*, and 8 rhesus, *Macaca mulatta*) in each of which a small lesion was made in the hypothalamus. In order to provide an adequate control series a careful examination was made of the gastro-intestinal tract of all primates sacrificed in this laboratory for a period of 6 months. These (some 50 in all) had been subjected to various operative procedures, including occlusion of the pituitary stalk, spinal cord transection and ablation of certain cortical areas, frontal, premotor and occipital.

Operative Procedure

A subtemporal approach was used throughout this investigation. This method was selected in order to avoid damage to any part of the central nervous system, other than the hypothalamic region, as the animals were also being used for anatomical studies of nerve pathways. All operative procedures were carried out with full aseptic precautions and under sodium amytal anaesthesia.

A large unilateral bone flap extending across the midline was turned down in each case. With the head of the animal placed on its side the temporal lobe was gently retracted upwards from the base of the skull, thereby exposing the optic and oculomotor nerves and the infundibular stalk partially screened by the internal carotid artery. By means of a sharp hooked probe a small lesion was made in a selected part of the hypothalamus, either directly in the tuber cinereum or in the posterior hypothalamic region. An attempt was made to produce a bilateral lesion in every case. Little haemorrhage was encountered, though frequently a small amount of cerebrospinal fluid escaped from the puncture. The temporal lobe was allowed to slip back into position, the bone flap replaced and the wound closed.

During the elevation of the temporal lobe there was often a marked slowing of the heart rate. In the earlier experiments, in order to combat this effect, a small dose (0.3 to 0.5 cc.) of adrenalin was administered subcutaneously at this stage of the procedure, but this was dispensed with in later experiments.

Postoperative Study

Most of the animals survived 3 days and were sacrificed at the end of this period as it was particularly desirable in this investigation to observe the early appearances of any gastric lesions. Two of the animals were sacrificed at the end of 3 weeks and were utilised for Marchi studies. During the postoperative period careful observations were made of the general condition of the animal and the faeces and any vomitus were examined for blood.

At the time of sacrifice the animals were profoundly anaesthetised with ether. The stomach and intestines were isolated from the general circulation, removed and placed in physiological saline for immediate examination. The animal was then injected through the

abdominal aorta with 500 to 1000 cc. of 0.9 per cent saline until clear fluid flowed from the opened inferior vena cava. This was followed by 300 to 500 cc. 10 per cent chloral hydrate solution. The brain and spinal cord were removed at once and a complete autopsy performed.

Histological Technique

The hypothalamus and adjacent brain tissue were removed in one piece and placed in 75 per cent alcohol. Serial Nissl-stained sections were made of the entire block so that the precise localisation of the lesion could be ascertained in each case. The rest of the brain stem and sample blocks from the spinal cord were stained with Cajal's method of reduced silver nitrate impregnation (formula 6a) and serial sections made for examination of degenerated *bouton terminaux* (as described by Hoff ⁷). Any lesions found in the gastrointestinal tract were excised and fixed in neutral formalin solution and sections were stained with haemalum and eosin.

RESULTS

Postoperative Course

The animals showed striking individual variations in the general postoperative condition. Some made a rapid recovery so that within 24 hours their behaviour was apparently entirely normal. Many of the animals, on the other hand, were profoundly affected by the operation. They recovered slowly from the anaesthesia and sat hunched up in the cage with the head lowered between the upper limbs. These animals refused food and invariably their condition became progressively worse, death supervening in 1 to 3 days.

Subsequent histological examination of the base of the brain revealed nothing in the location or extent of the lesion that would account for the marked difference in postoperative recovery. Thus, in Experiment 7, in which serial sections of the hypothalamus showed a bilateral lesion that had destroyed the tuber cinereum, opened up the base of the third ventricle and had all but severed the infundibular stalk, the animal was active and feeding several hours after completion of the operation. It continued to improve so that on the following morning its general behaviour and activity were indistinguishable from those of an unoperated animal. Sacrifice 3

days after the operation revealed no abnormality in the gastro-intestinal tract. Contrasting remarkably with this case was the postoperative course in Experiment 5 in which the lesion in the hypothalamus was non-haemorrhagic and identical with that produced in Experiment 7. Yet the animal went steadily downhill and died within 24 hours of the operation. Autopsy in this case revealed multiple mucosal erosions in the body of the stomach. It would appear from our experiments that extensive gastro-intestinal lesions may alter very rapidly the postoperative course of these animals, quite apart from the nature of the hypothalamic lesion produced.

In only 1 case was haemorrhagic vomitus observed. In Experiment 11, which subsequently showed a destructive lesion in the dorsal part of the tuber cinereum, the animal remained prostrate for 24 hours after the operation. Twenty hours after the lesion had been made the monkey began to vomit a clear mucous fluid mixed with clots of coffee-coloured blood. During the next few hours the animal recovered slightly but continued to vomit blood at intervals, and death occurred within 48 hours of operation. At autopsy multiple haemorrhagic erosions were found in the body of the stomach.

A marked polydipsia following operation was observed in several instances but as the animals were being sacrificed at the end of 3 days no reliable measurements of the daily fluid intake and output were obtained.

Occurrence of Gastric Erosions

Three of the 19 animals died while the lesion was being made. Of the 16 that survived the operation 5 animals showed acute mucosal erosions in the stomach. Two of these survived a 3 day postoperative period, although exhibiting a marked disinclination to eat and a general sluggishness in movements. The remaining 3 of the group showing gastric erosions at autopsy died, 2 within 1 day and 1 in 2 days, each after a stormy postoperative course.

In all of the cases the erosions were confined to the body of the stomach, both the anterior and posterior wall. Sometimes the pyloric region was also involved but there was no evident predilection for this part, or for the lesser curvature of the stomach. The erosions were always multiple and haemorrhagic (Fig. 1) and there were often clots of dark blood intermingled with the gastric contents, definitely establishing the ante mortem nature of such lesions. Some

of the erosions were 5 mm. by 5 mm. and showed a punched-out appearance with slightly raised margins and a haemorrhagic base (Fig. 2), but all were evidently of very recent origin. Microscopical examination revealed that the lesions were all confined to the mucosa and in no case was the muscular layer involved. The histological picture was one of sharply localised destruction of the mucosa with haemorrhagic exudate and no evidence of thrombosis in the sub-mucosal vessels underlying the lesion.

In three of the five experiments in which gastric erosions were present the stomachs of the animals showed considerable dilatation and atony on opening the abdomen. In another animal, in which the lesion had involved the posterior hypothalamic region and yet showed no pathological changes in the gastro-intestinal tract, a similar condition of the stomach was observed at autopsy. This marked dilatation of the stomach might conceivably be attributed to sympathetic hyperactivity. However, we have since seen similar very dilated stomachs in several animals sacrificed after various kinds of cerebral but non-hypothalamic operations, and its significance is therefore not entirely clear.

In the control series of over 50 animals subjected to many types of non-hypothalamic cerebral lesions and sacrificed in this laboratory during the past 6 months, careful postmortem examination of the gastro-intestinal tracts has revealed only 1 case with gastric or duodenal ulceration. This animal was a young capuchin in which the right and left motor and premotor areas had been extirpated 5 months previously. Five weeks before autopsy a transection of the spinal cord at the sixth thoracic level had been performed and 12 days prior to sacrifice the left posterior quadrant of the spinal cord had been divided at the third cervical level. At autopsy the stomach showed three small haemorrhagic erosions, one close to the oesophageal orifice and two in the pyloric end. The contents of the stomach were mucous and mixed with small particles of black blood. The small and large intestine were normal.

This case, in which interruption of sympathetic or parasympathetic pathways from higher centres undoubtedly played a significant rôle in the development of the gastric erosions, stands out as an isolated example from a large series of monkeys that had been subjected to many and varied types of non-hypothalamic cerebral injury and revealed no gastro-intestinal pathology at autopsy. The

consistently negative findings in the long control series of experiments continued from Watts' and Fulton's investigation, lend greater significance to the association of gastro-intestinal lesions with injury to the hypothalamus or interruption of its descending autonomic pathways.

We were impressed at autopsy of primate material with the frequent finding in the large and small bowel, and in one instance in the mucosa of the stomach, of lesions of parasitic origin which often closely simulated chronic peptic ulceration, and it was sometimes necessary to resort to histological methods in order to differentiate the conditions. Tuberculous ulceration of the intestine was also encountered in several instances, but it would seem that true peptic ulceration is a very rare occurrence in normal monkeys.

Site of Lesions in the Hypothalamus

Serial sections showed that in most of the cases the lesions had been made in the tuber cinereum. In 3 cases the posterior hypothalamic region only had been damaged, and in 1 case the lesion had destroyed the supra-optic nucleus on one side. The pre-optic region was never involved.

Of the 5 animals showing well marked gastric erosions at autopsy, all showed lesions confined to the infundibulum or to the grey matter immediately dorsal to it, in which lie the nucleus hypothalamicus periventricularis (anterior and posterior) and nucleus hypothalamicus ventro-medialis* of Papez and Aronson.⁸ The nuclei tuberis lateralis were destroyed in all 5 cases, and in 2 animals there was considerable involvement of the pars tuberalis of the pituitary body, which in the monkey wraps round the base of the infundibular stalk and is in part embedded in the substance of the tuber cinereum. In none of the 5 cases did the lesion encroach upon the nucleus paraventricularis, nor was the nucleus mammillo-infundibularis (hypothalamicus lateralis of Papez and Aronson⁸) involved to any extent. In only 1 of the 5 was the track of the lesion haemorrhagic, and in all 5 cases the base of the third ventricle had been opened.

The precise localisation of the lesion in the 5 cases showing gastric erosions can be summarised briefly as follows.

* The ventral or "principal" nucleus of the tuber cinereum.

EXPERIMENT 4. *Operation January 19, 1934; Slow Postoperative Recovery; Disinclination to Eat; Sacrifice 3 Days Later; Three Small Ulcers in the Body of the Stomach*

Brain Lesion: In sections through the optic chiasma the supra-optic and paraventricular nuclei on each side contained many "shadow" cells, pale and partly disintegrating, and there was a fairly extensive gliosis present. In more caudally placed sections the oral part of the tuber cinereum appeared intact and there was no haemorrhage to be seen. At the point of origin of the infundibular stalk the base of the third ventricle was widely opened from below, the stalk apparently having been separated completely from the base of the brain by the injury. There was no evidence of haemorrhage anywhere. The track of the lesion could not be traced, owing to the destructive nature of the injury, but it appeared to have been uniformly bilateral and the tuber cinereum, including the nucleus tuberis on each side, had been completely destroyed. The posterior hypothalamic region and corpora mammillare were intact.

EXPERIMENT 5. *Operation January 26, 1934; Death on the Following Day; Multiple Gastric Erosions*

Brain Lesion: The track of the lesion could be seen entering the tuber cinereum from the right side, immediately rostral to the base of the infundibular stalk. There was very extensive destruction of the tuberal tissue at this point and the base of the third ventricle was opened. The lesion passed across to the opposite side, but was much more extensive on the right. There was no haemorrhage to be seen in any of the sections. The injury was confined to the nucleus tuberis and the grey matter in the floor of the third ventricle in the immediate vicinity of the tuber cinereum. The supra-optic, paraventricular and mammillo-infundibular nuclei were not involved and the posterior hypothalamic region was intact.

EXPERIMENT 9. *Operation February 5, 1934; Death on the Following Day; Large Dilated Stomach with Multiple Mucosal Erosions*

Brain Lesion: The track of a circumscribed haemorrhagic lesion could be traced, entering the lateral aspect of the tuber cinereum from the left side, just rostral to the commencement of the pituitary stalk. The base of the third ventricle was opened and there was

considerable damage to the right lateral wall. A clot of blood overlapping the optic chiasma extended backwards on each side of the infundibular stalk. There was also a small haemorrhage in the cavity of the third ventricle, around the lacerated right lateral wall. The damage was confined to the tuber cinereum bilaterally and involved the pars tuberalis of the hypophysis. The supra-optic, paraventricular and posterior hypothalamic nuclei were intact.

EXPERIMENT 11. *Operation February 8, 1934; Death Within 48 Hours; Dilated Atonic Stomach with Multiple Mucosal Erosions*

Brain Lesion: The lesion was very similar to that in Experiment 9, but had been placed more dorsally. The instrument had entered the hypothalamus on the right side above the tuber cinereum and had pierced the right lateral wall of the third ventricle without much involvement of the opposite side. There was considerable tissue destruction but no demonstrable haemorrhage along the track of the lesion. The grey matter in the floor of the third ventricle, comprising the nuclei hypothalamicus periventricularis et ventro-medialis, had been extensively destroyed but the origin of the pituitary stalk was relatively intact. The nucleus mammillo-infundibularis on the right side was damaged but the rest of the hypothalamic nuclei were uninvolved.

EXPERIMENT 15. *Operation February 26, 1934; Good Postoperative Recovery; Polydipsia and Disinclination to Eat; Sacrifice 3 Days Later; Multiple Haemorrhagic Erosions in the Body of the Stomach*

Brain Lesion: The lesion was entirely unilateral and confined to the oral parts of the tuberal region. The supra-optic nucleus and the nuclei hypothalamicus periventricularis et ventro-medialis on the left side had been damaged; there was no involvement of the mammillo-infundibular nucleus or the posterior hypothalamic region. No evidence of haemorrhage could be seen in any of the sections.

DISCUSSION

Positive evidence is brought forward here to show that in monkeys histologically verified lesions, *confined to the tuberal nuclei*, leaving all other hypothalamic nuclei intact, may lead to haematemesis and

multiple mucosal erosions in the body of the stomach. Several other cases with brain lesions, identical in localisation and extent, were unassociated with any recognisable gastro-intestinal pathology. It would appear that an irritative process caused by haemorrhage at the site of the hypothalamic injury is not a deciding factor in the production of gastric erosions, since such haemorrhage in the hypothalamus occurred in only 1 out of the 5 cases showing erosions, and since several cases with extensive haemorrhagic lesions revealed no evidences of pathology in the gastro-intestinal tract. In three experiments in this series with lesions confined to the posterior hypothalamic region, at autopsy no erosions were found in the stomach, but the cases are too few to warrant any statement regarding the relation of the posterior hypothalamic nuclei to gastric ulcer.

In the series of Watts and Fulton⁶ the injury in most cases involved the tuberal, supra-optic and paraventricular nuclei and, as in our series, no case of gastric erosions was encountered in association with posterior hypothalamic injury. In the experiments of Burdenko and Mogilnitzki⁴ the injury was made immediately behind the infundibular stalk, and in those of Keller, Hare and d'Amour⁵ at the level of the optic chiasma. The association of tuberal lesions with gastro-intestinal ulceration seems therefore established.

Two interpretations have been advanced in the consideration of the neurogenic factor in the genesis of experimental gastric ulcer, one based on a conception of sympathetic hyperactivity, the other explaining the phenomena on grounds of parasympathetic hyperactivity.

(1) *Sympathetic Hyperactivity*

The Peripheral Mechanism: According to this view the sequence of events which leads to the production of gastric ulceration is believed to be overactivity of the sympathetic vasoconstrictors, spasm of the terminal vessels in the submucosa of the gastro-intestinal tract, with the production of multiple areas of ischaemia in the mucosa and digestion of the necrosed tissue by the acid gastric secretions, leaving small punched-out erosions.

The Central Mechanism: In order to explain on the basis of this theory the association of erosions with hypothalamic injury, it is

necessary to consider either a "release" or an "irritation" of the sympathetic centres in the hypothalamus. The development of erosions within 24 hours of the brain injury can be interpreted equally well on the basis of an irritative or of a release mechanism, either of which can be assumed to exist immediately following the injury. It is, however, rather difficult to understand how the small lesions in the present series, which were confined to the tuberal nuclei, could have interrupted any great number of inhibitory fibres from higher centres.

(2) *Parasympathetic Overactivity*

The Peripheral Mechanism: Cushing¹ has summarised the possible mechanism by which hyperactivity of the parasympathetic apparatus may act in the production of gastric ulceration as follows: "Direct stimulation of the tuber or of its descending fibre tracts, or what theoretically amounts to the same thing, a functional release of the vagus from paralysis of the antagonistic sympathetic fibres, leads to hypersecretion, hyperchlorhydria, hypermotility and hypertonicity especially marked in the pyloric segment. By the spasmodic contractions of the musculature, possibly supplemented by accompanying local spasms of the terminal blood vessels, small areas of ischaemia or haemorrhagic infarction are produced, leaving the overlying mucosa exposed to the digestive effects of its own hyperacid juices."

The Central Mechanism: Hypothalamic lesions may lead to overactivity of the parasympathetic system either by direct destruction of the sympathetic centres, thus releasing the vagus from the antagonistic sympathetic activity, or by irritation of parasympathetic pathways in this part of the brain. Beattie and Sheehan⁹ showed that faradic stimulation of the anterior part of the hypothalamus in the fasting cat resulted in parasympathetic phenomena, *i.e.* in a rise of intragastric pressure which was followed by an increase of peristaltic movements of the stomach.

The lesions associated with gastric erosions in the present series of experiments were confined to the tuberal nuclei and it is difficult to see how they could have caused any marked destruction of the sympathetic centres which extend backwards into the posterior hypothalamic region. If the anterior hypothalamic region can be assumed tentatively as the location of parasympathetic discharge (Cushing,¹

Beattie¹⁰), then the evidence here is suggestive of an irritation of such parasympathetic pathways as the underlying mechanism, since all the lesions were small and superficial in extent. Functional localization in the hypothalamus is however not sufficiently clarified to warrant any final conclusion from the present study as to the exact mechanism involved. A study of the experimental production of gastric ulcer following *peripheral* nerve lesions shows that splanchnotomy is by far a more frequent cause than vagotomy (Cushing¹) although vagal section has led to acute ulceration in the stomach and duodenum in some cases (Ferguson¹¹).

We have attempted to throw more light on the question by a pharmacological approach to the problem. For this purpose monkeys (*Macaca mulatta*) have been subjected to repeated subcutaneous injections of (a) acetyl- β -methylcholine chloride in amounts of 25 to 50 mg. per kg. body weight, or (b) adrenalin 0.5 cc. of a 1:1000 solution per kg., or (c) adrenalin 1.5 cc. of a 1:1000 solution per kg., with atropine 20 mg. per kg., or (d) pituitrin (surgical) 1 to 3 cc. (The injections of pituitrin were given in view of Dodds, Noble and Smith's¹² recent findings in rabbits.) The doses were given in each experiment every 2 to 3 hours for periods of 3 to 7 days. In no case were there observed at autopsy any erosions of the gastric mucosa comparable to the results obtained from hypothalamic injury. Although these negative findings cannot be accepted as conclusive evidence, and the cases are too few in number, nevertheless they indicate that states of profound overactivity of the sympathetic or of the parasympathetic system may exist for comparatively prolonged periods without the appearance of gastric erosions.

In considering the neurogenic influences in the production of gastro-intestinal lesions it must not be lost sight of that a vasomotor disturbance is only one of many factors that undoubtedly plays an important rôle. The motility of the gastro-intestinal tract and the amount and nature of the digestive juices are under the direct influence of the central nervous system. The influence of the vagus nerve on gastro-intestinal motility is usually looked upon as due to an augmentation of peristalsis and an inhibition of sphincteric action, while the sympathetic innervation to the gut is considered to act in an antagonistic manner. McSwiney¹³ considers, however, that it is possible to postulate the presence of motor and inhibitory fibres to the stomach in both the vagus and sympathetic nerves, and

that the immediate effects of vagotomy and splanchnotomy are similar, namely retardation of function. It is highly probable therefore that there is no real fundamental opposition in the two views that have been advanced to account for the occurrence of gastrointestinal lesions of nervous origin, and that the explanation may lie in a complex imbalance between the sympathetic and parasympathetic systems.

SUMMARY AND CONCLUSIONS

1. In 16 monkeys, following hypothalamic injury, 5 animals showed multiple haemorrhagic erosions in the mucosa of the body of the stomach.

2. The animals showed striking individual variations in the general postoperative condition. Those with gastro-intestinal lesions showed a disinclination to eat, their condition became progressively worse, and in three experiments death supervened in 24 to 48 hours.

3. In all of the cases the erosions were confined to the stomach, none occurring in the duodenum. The erosions were multiple and haemorrhagic, entirely confined to the mucosa, and some showed a punched-out appearance.

4. In three of the five experiments in which gastric erosions were present the stomachs showed considerable dilatation and atony, suggestive but not conclusive evidence of sympathetic activity.

5. Histological examination of the hypothalamic injuries revealed that in all the animals showing gastric erosions at autopsy the lesions were small and confined to the tuberal nuclei. In only 1 of the 5 was the track of the injury haemorrhagic. Positive evidence is therefore advanced to show that histologically verified lesions, confined to the tuberal nuclei and leaving all other hypothalamic nuclei intact, may lead to haematemesis and multiple mucosal erosions in the body of the stomach.

6. In a control series of over 50 monkeys subjected to many and varied types of non-hypothalamic cerebral lesions careful autopsy examination of the gastro-intestinal tracts revealed only 1 case with gastric or duodenal ulceration, and this animal had been subjected to a bilateral motor and premotor extirpation 5 months prior to sacrifice and to transections of the spinal cord at the sixth thoracic and third cervical levels 5 weeks and 12 days (respectively) before autopsy. The consistently negative findings in the control experi-

ments would appear to lend greater significance to the association of gastro-intestinal lesions with injury to the hypothalamus or interruption of descending autonomic pathways.

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DESCRIPTION OF PLATES

PLATE 113

FIG. 1. Drawing to show the naked eye appearance of the mucosal surface of the stomach when first opened. The erosions are multiple and haemorrhagic. (Experiment 9. Capuchin. Death within 24 hours after a hypothalamic lesion in the tuber cinereum.)

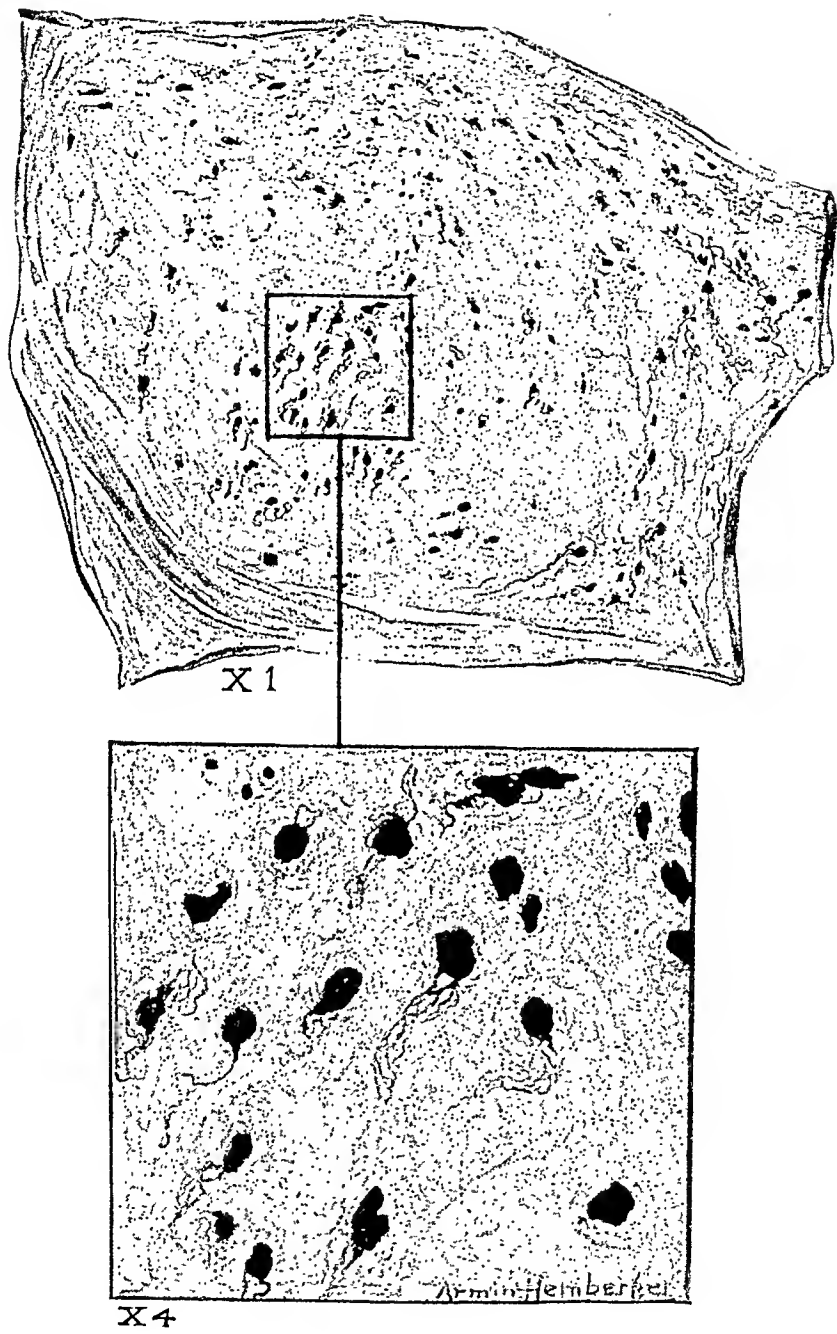
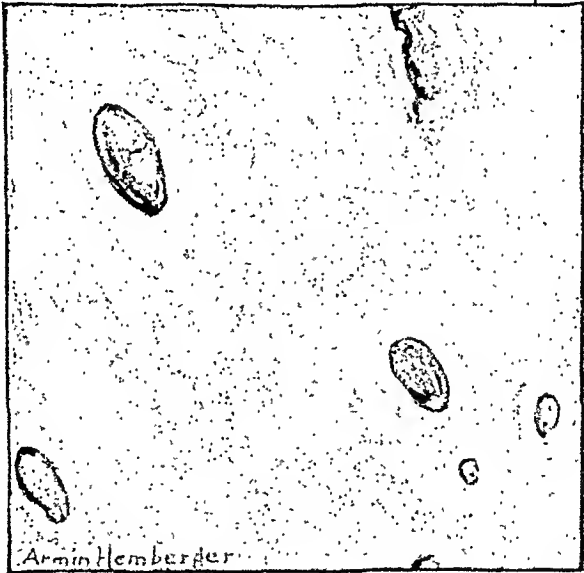
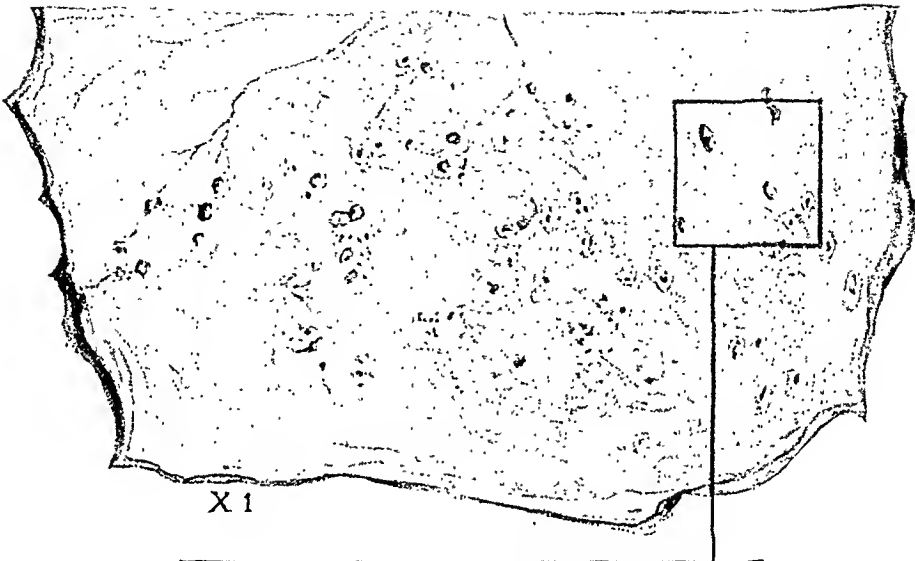


PLATE 114

FIG. 2. Drawing to show the punched-out appearance of the gastric erosions, the bases of which are haemorrhagic. All were of recent origin and occurred chiefly on the dorsal wall of the stomach, extending from the oesophageal opening to about 2 cm. from the gastroduodenal junction. (Experiment 5. Capuchin. Death within 24 hours after a hypothalamic lesion confined to the tuberal nuclei.)



X4

FUNCTIONAL COR TRILOCULARE BIATRIA *

REPORT OF A CASE WITH A MALPOSITION OF THE SEPTUM IN THE VENTRICLES

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Cases of malposition of a defective septum in the ventricles, so that both atriums empty into a common chamber, appear to form a definite entity. Twelve cases have been reported in the literature by Holmes,¹ Peacock,² Favorite,³ von Rokitansky⁴ (2 cases), Thérémín,⁵ Chiari,⁶ Mann,⁷ Marchand,⁸ Mills⁹ and Spitzer¹⁰ (2 cases). These hearts have been designated as cases of functional cor triloculare biatria. In all these cases the septum was placed to the right, separating a small right ventricle from a large left ventricle in which both auriculoventricular orifices were present. In the first case reported (Holmes¹) the pulmonary artery arose from the small right chamber. In the remaining cases the aorta arose from the latter in transposed relation to the pulmonary artery. The writer has had the opportunity to study a heart which is unique in that the septum was displaced to the left instead of to the right. The aorta arose from the small left chamber and was in a transposed relation to the pulmonary artery, which sprang from the large right ventricle. The right chamber contained both venous orifices. With certain associated anomalies this heart produced a complex picture, and an attempt will be made in this paper to trace its development in the light of recent researches on the embryology of the heart.

REPORT OF CASE

Clinical History: Baby girl, K. M., aged 5 months, was admitted to the Pediatric Service of the Brooklyn Jewish Hospital on Nov. 1, 1931, with a history of malnutrition since birth, cough and coryza of 1 weeks duration, and cyanosis of the lips for 2 days.

The mother of the child was 30 years of age and in good health. She had one other child, aged 7 years, living and well. Following the birth of this baby, she had three miscarriages, the first two at 3 months and the third at 4 months. She then gave birth to a full term infant who had a large head and died in convulsions 5 hours postpartum.

* Received for publication March 6, 1935.

The patient was born spontaneously at full term. There was no history of any birth injury but she was markedly cyanotic at birth and was resuscitated with difficulty. Her birth weight was 7 pounds 4 oz. She was ill since birth and failed to gain weight, taking all her feedings poorly, each of which was vomited, the vomiting sometimes being projectile in type. When the baby was 2 months of age a physician said she had an enlarged heart, which was confirmed by fluoroscopy. She always had very cold extremities. Two weeks before admission she developed a cough and coryza which persisted, but cyanosis, absent since birth, did not reappear until 2 days before admission.

On admission, examination revealed an acutely ill, marasmic infant, slightly cyanotic. A mucopurulent discharge from the nose was present and the pharynx was very congested. There was a diminished percussion note over the left chest. Bronchial breathing and numerous crepitant râles were present in several parts of the lung. The right border of the heart was percussed at its farthest point, 3 cm. from the midline, and the apex 1.5 cm. to the left of the left midclavicular line in the fifth interspace. The apex beat was diffuse and heaving, and a palpable systolic thrill was present over the entire precordium, but most marked at the apex. The heart sounds were of good quality, rapid and regular. A loud, moderately high pitched, rough systolic murmur was heard over the entire precordium, being most marked at the apex. The spleen and liver were not palpable. The extremities were very cold. No deformities were present. A clinical diagnosis of congenital heart disease, acute nasopharyngitis, acute bronchopneumonia, and marasmus was made. A blood transfusion was given, but the child grew progressively worse and died Nov. 13, 1931, after 12 days in the hospital. The temperature throughout remained normal and subnormal, except for an ante mortem rise. Cyanosis was never marked.

The blood count was 3,880,000 erythrocytes, 20,500 leukocytes, 76 per cent polymorphonuclear neutrophils and 22 per cent lymphocytes. The hemoglobin was 58 per cent (Dare). The urine showed 1 plus albumin. Tuberculin, Schick, Wassermann and Kahn tests and nose and throat cultures were negative.

X-rays of the chest revealed a bronchopneumonia. The cardiac shadow was reported as broadened slightly to the right and considerably to the left, the general condition suggesting the presence of congenital heart disease. There was no evidence of luetic changes in the X-ray plates of the extremities.

Electrocardiographic studies, unfortunately, were not made.

AUTOPSY REPORT

The body is that of a well developed, emaciated female infant of 6 months, 60 cm. in length, weighing 7 lbs. and 9 oz. The skin is slightly cyanotic. The thymus weighs 2 gm. and appears normal on section. Both lungs show numerous, well demarcated patches of reddish brown color which microscopically show exudation of fluid, fibrin, red blood cells and leukocytes into the alveoli. The remaining organs show varying degrees of congestion and cloudy swelling of the parenchyma.

Heart: The heart weighs 4 gm. Notes on the position of the heart

in the body are lacking. Viewed anteriorly, the organ appears as a right angle triangle with the right angle in the upper left portion where the aorta emerges. The left border of the ventricle, 6 cm. long, descends almost vertically while the right border, 7.5 cm. long, curves downward from right to left forming the hypotenuse. The heart appears rather pale red in color, is firm in consistence, and there is a diminished amount of fat in the auriculoventricular sulcus. The visceral and parietal pericardiums are smooth and glistening, and no defects or adhesions are present. There is a normal quantity of clear fluid in the pericardial sac.

The atriums are present in their normal position, and their appendages curve normally on either side of the great vessels. The largest circumference of the right atrium is 4 cm. The entrances of the superior vena cava, inferior vena cava and the coronary sinus are in their normal relations, and no anomalies of the venous valves are present. There is a slight valvular patency of the foramen ovale. The left atrium is 4.5 cm. in its largest circumference and shows no pathological changes. It receives four pulmonary veins in their normal relations.

On opening the ventricles posteriorly, it can be seen that the septum is displaced to the left, dividing approximately the right four-fifths of the heart from the left one-fifth, producing a small chamber on the left and anteriorly (which gives off the aorta) and a large chamber on the right and posteriorly. In the latter, both auriculoventricular orifices and the orifice of the pulmonary artery are present (Fig. 1).

The ventricle of the left side is reduced in size to a small cone 1.5 cm. long, the base of which is occupied entirely by the root of the aorta which is 2 cm. in circumference. The trabeculae on the anterior wall are prominent. Those posteriorly are flattened and the septal wall is smooth.

The ventricle on the right side is about four times the size of that on the left and is also conical in shape. The myocardium measures 1 cm. at its thickest portion near the base posteriorly and is thinned to 4 mm. near the apex. There can be distinguished two papillary muscles in close approximation on the anterior wall, one on the lateral, and two on the posterior wall. On the septal wall there is a low band of muscle 0.5 cm. wide and 2 cm. long, continuous with the aortopulmonary septum, and coursing downwards, anteriorly,

toward the right, to end in the anterior wall. The remaining portions of the inner aspect of the ventricular walls are traversed by fine interlacing strands of muscle and fibrous tissue, except for the septal surface which is smooth.

The auriculoventricular orifice on the right side is 3.5 cm. in circumference and is occupied by a large medial cusp, the base of which is attached to the free edge of the interauricular septum, and a long, narrow, lateral cusp in which individual cusps cannot be identified. The medial cusp is attached anteriorly to the medial of the two anterior papillary muscles, and posteriorly to the muscle on the lateral wall. The lateral cusp is attached to both the two posterior and the lateral muscles. On the left side there is also a medial and a lateral cusp. The former is attached to the free edge of the interatrial septum on a base in common with that of the medial cusp of the right side. Posteriorly the former cusp is connected to the right posterior papillary muscle, while anteriorly it is joined to the superior wall of the septal defect (the aortopulmonary septum) by short chordae. The lateral cusp is similarly attached anteriorly, while posteriorly it is connected to the large, left, posterior muscle.

The septum (Fig. 1) is roughly triangular in shape, 2.5 cm. wide at the base, 5 cm. long, 0.5 cm. thick, and is curved slightly from left to right, so that its concavity looks to the right and posteriorly. Its apex is on the left border of the heart about 1.5 cm. from the apex of the heart. The base is attached to the ventricular walls by an anterior and a posterior root. The former is attached to the right auriculoventricular orifice at the anterior end of the common base of the medial cusps of both auriculoventricular openings. The posterior root is attached to the posterior ventricular wall, caudal and to the left of the anterior attachment. Between these two roots the base of the septum forms a free falciform edge. The aortopulmonary septum from above meets this edge in the following way: anteriorly the former is in a plane slightly more anteroposterior than that of the interventricular septum, and thus its attachment to the anterior ventricular wall is to the left of the anterior root of the septal base. A triangular depression (Fig. 1) is thus formed on the anterior ventricular wall between the aortopulmonary septum on the left and the anterior root of the interventricular septum on the right. The orifice of the pulmonary artery is bounded anteriorly by the base of this triangular depression, and posteriorly by the common base of

the medial auriculoventricular cusps. Posteriorly the aortopulmonary septum is the plane of the interventricular septum, but fails to join with it, resulting in a defect 1.2 cm. in diameter which connects both ventricles. This defect is roughly diamond-shaped and completely surrounded by muscle, above by the aortopulmonary and below by the interventricular septum.

The pulmonary orifice (Fig. 1) is to the right and posterior to the aortic orifice. The great vessels maintain this relation as they course distally. No torsion is present. Thus, distally they are in their normal relation, while proximally their normal positions are reversed.

The semilunar cusps are normal in size, shape and number. The non-coronary cusp is placed anteriorly.

The coronary arteries and veins are not injected, and only the larger vessels can be distinguished. The right coronary artery emerges from the right aortic sinus and gives off immediately in the auriculoventricular sulcus a large branch, which descends toward the left on the anterior surface of the heart to the apex. Near its origin this branch gives rise to another branch which descends toward the right, dividing into several tributaries. The artery continues in auriculoventricular sulcus around the right border of the heart, giving off in its course several short branches which descend toward the apex.

The left coronary artery arises from the left aortic sinus and courses toward the left in the auriculoventricular sulcus for 1.5 cm. where, at the junction of the left atrium and its appendage, it gives off a large branch which descends on the posterior surface toward the left border and the apex. It divides into several branches near the apex, which course around the left border to mingle with the branches of the anterior descending artery. The remaining small portion of the artery courses in the auriculoventricular sulcus toward the right coronary, giving off several short descending branches. No abnormality was noted in walls of the vessels.

The coronary sinus courses posteriorly in the auriculoventricular sulcus, toward the left border.

Microscopic examination of the heart muscle revealed no lesions. No attempt was made to trace the course of the conduction system.

DISCUSSION

Let us first direct attention to the corrected transposition of the great vessels that exists in this heart. By corrected transposition of the main trunks is meant that condition in which the two vessels exist in reverse relation to each other (the aorta being placed anteriorly instead of posteriorly in relation to the pulmonary artery) yet spring from the ventricles to which they normally belong. Examples of this condition are of rare occurrence, there being only 21 reported in the literature. Robertson,¹¹ Sato¹² and Spitzer¹⁰ have accounted for their occurrence by supposing that a situs inversus of the ventricles occurs with a transposition of the great vessels (the atriums remaining in their normal position), so that the right or tricuspid ventricle is formed on the left side but still gives rise to the aorta; and similarly, the left or bicuspid ventricle forms on the right side and gives rise to the pulmonary artery. Von Rokitsky⁴ labelled these cases as corrected transposition of the great vessels, since he did not draw the distinction between bicuspid and tricuspid ventricles, calling a ventricle on the right side the right ventricle, even though it had a bicuspid valve and no conus. He believed that the correction was due to a sympathetic adjustment of the interventricular septum with the bulbar septum, so that the great vessels opened into their proper ventricles. However, the view that corrected transposition is produced by an independent situs inversus of the ventricle loop seems well established, especially since in the reported cases there is characteristically a reversal of the auriculoventricular cusps and an absence of the conus arteriosus in the right ventricle. In a heart described recently by Walmsley¹³ there is additional evidence of an independent situs inversus of the ventricles besides a reversal of the auriculoventricular cusps which, alone, cannot be taken as good evidence. Walmsley lists, in describing his heart, a reversal of the internal conformation of the ventricles, a reversal in the forms of the auriculoventricular bundle, a reversal of the coronary artery fields, and a left-sided position of the interventricular septum, so that the pars membranacea is placed between the left atrium and right ventricle, instead of between the right atrium and left ventricle. Thus, because of the presence of a corrected transposition of the great vessels, it is believed that a ventricular situs inversus exists in this heart. Additional evidence

of this condition will be seen in studying the other existing malformations.

Let us turn now to a consideration of the development of the septum on the right side, as was present in the reported cases of functional cor triloculare mentioned above. Cases have been described in which there was also a septum separating the conus from the sinus of the right ventricle. This septum is explained by Keith¹⁴ as a persistence of the lower bulbar orifice. It exists ordinarily in reptiles. It usually shows much fibrous thickening in the human heart, and so it has been thought to be due here to inflammatory contraction of the conus. However, the septum may be muscular, as in a case reported by Bohm,¹⁵ in which the sinus was separated from the conus by a muscular ridge. This septum may also exist with an interventricular septum, as in the 2 cases reported by Mackenzie¹⁶ of hearts with "three ventricles," and in a case reported by Dudzus¹⁷ of a heart with "four ventricles." In the hearts with functional cor triloculare reported by Young¹⁸ and Peacock,² in addition to the right-sided septum, a low muscular ridge was present in the normal position of the interventricular septum. Thus, these authors considered that the right-sided septum represented the persistent lower orifice of the bulbus cordis and had grown entire, separating the two portions of the right ventricle, while the development of the interventricular septum had been arrested. Spitzer¹⁰ believed that the right-sided septum in his cases, called by him "mixed transposition" of the great vessels, was a septum spurium formed by the fusion of the hypertrophied crista supraventricularis and the anterior tricuspid ridge, while the true interventricular septum was rudimentary or entirely absent. His theory fails to account for the condition in the heart reported by Holmes, mentioned above, in which the malposition of the septum was present without a transposition of the vessels.*

Abbott¹⁹ made a study of the heart reported by Holmes and a similar heart in the museum of the Harvard Medical School, and came to the conclusion that, in these specimens at least, the strong muscular wall with a large defect at its upper border through which

* Furthermore, as Walmsley points out, in corrected transposition of the vessels Spitzer considers the anterior part of the interventricular septum to be formed from the crista supraventricularis, although the latter should be formed posteriorly on account of the presence of the situs inversus of the ventricles, which Spitzer believes exists in these cases.

the small cavity communicated with the large common ventricle, was simply the malposed interventricular septum. Similarly, in the heart reported here, that the septum was the true interventricular septum is strongly evidenced by the fact that it was a strong muscular wall with a free falciform edge at its base which, anteriorly, was in relation to the anterior endocardial cushion, and posteriorly to the right bulbar ridge, the conditions closely resembling those present in the embryo (Fig. 2). It is evident that the malposition was either due to a growth of the septum primarily in its position to the right of the right auriculoventricular orifice, or else was brought there in the development of the heart from its normal or abnormal position.

Abbott considered that a septal defect, produced by failure of union of the aortic with the interventricular septum, would, as she stated in 1901,²⁰ bring the blood of both atriums to the left ventricle which, thus having an increased amount of work, would enlarge greatly and bring the defective septum, in the further development of the heart, to the right. It is not clear from this description why the septum should be displaced to the right side of the tricuspid orifice from the left side where it was attached. Interventricular septal defects are relatively common, yet they are rarely associated with displacement of septum to the right of the tricuspid orifice, and never with sufficient enlargement of the left ventricle to bring it there. One cannot conceive how, in view of the dynamics of the circulation, the interventricular septum can be brought across a venous orifice in the development of the heart.

In view of these considerations it is necessary to assume that the alternative occurs, namely that the septum develops primarily to the right of the right auriculoventricular orifice. Normally in the early stages of the development of the heart the posterior border of the septum becomes closely attached to the left side of the right auriculoventricular opening (Fig. 2), but if for some reason it had grown only slightly farther to the right it would have been brought into relation with the right bulbar ridge on the right side of the auriculoventricular opening. Then if development continued the usual way, the bulbar ridges fusing with each other and with the interventricular septum, the right auriculoventricular ostium would remain in the left ventricle along with the mitral orifice, and there would be present on the right side a small chamber without a venous orifice. In most of the reported cases of this condition detailed de-

scriptions are unfortunately lacking of the region of the attachments of the interventricular and aortopulmonary septums, and little evidence can be adduced from them in support of the above hypothesis.

The anatomical configuration of the heart reported here indicates that the position of the interventricular septum has been the result of a process such as that described above, but in the presence of a situs inversus of the ventricles. The anterior attachment* of the base of the interventricular septum remains in its normal position at the anterior end of the common base (Fig. 1) of the median cusps which represents in this heart the fused primitive auriculoventricular cushions. However, the posterior attachment, instead of being to the right of the left auriculoventricular orifice in relation to the posterior end of the common base of the median cusps, *i.e.* the primitive posterior endocardial cushion, becomes placed to the left of the left venous ostium joining with the posterior bulbar ridge.† The falciform edge which forms the inferior border of the primitive interventricular foramen can be traced in this heart between these two attachments (Fig. 1), so that the foramen resembles its early form except that, due to the left side displacement of the posterior attachment, it is in a plane placed obliquely from right anterior to left posterior, instead of the almost sagittal plane in which it is normally placed. The aortopulmonary septum, due to its malrotation, becomes placed in a plane more anteroposteriorly than the septum, so that the anterior ridge becomes attached to the left of the anterior root of the interventricular septum, instead of coming in direct relation to it (Fig. 1). Thus, there exists a condition which represents an arrest in development from the embryonic heart, *i.e.* the blood passes over the base of the interventricular septum and in front of the fused endocardial cushions to reach the pulmonary channel, which normally would be the aortic channel. The remaining portions of the aortopulmonary and interventricular septa fail to fuse, so that the two bulbar ridges superiorly, and the interventricular septum inferiorly, form the roughly diamond-shaped opening between the ventricles (Fig. 1), as described in the heart reported by Holmes. In this way these anatomical peculiarities which exist

* This would correspond to the posterior portion of the septum with the ventricles in their normal position.

† This would be more clearly conceived if one looks at Fig. 2 through a mirror or from the reverse side of the page.

in the heart reported in this paper are explained on a basis of a primary attachment of the posterior portion of the interventricular septum to the left of the left auriculoventricular orifice (instead of to the right of the right orifice if it had taken place in the normal position of the ventricular loop).

The median tendon or papillary muscle which, according to Mall²¹ is the most constant attachment of the tricuspid valve, is present in this heart as the connection between the medial and lateral cusps of the left valve with the aortopulmonary septum. This valve thus corresponds to the tricuspid valve of normal hearts, even though only two cusps can be distinguished. In independent situs inversus of the ventricular loop the auriculoventricular orifices remain in their normal position along with the atria, but since the connection of the median tendon is brought between the left valve and aortic septum, this valve instead of the right one is transformed into a tricuspid valve. The determination of the papillary muscles in this heart is difficult in view of the changes in their position and conformation brought about by the formation of a common ventricle. The two papillary muscles on the posterior wall and muscle on the lateral wall possibly represent, as is seen from their attachments to the right auriculoventricular valve, the anterior and posterior papillary muscles respectively of the normal heart, which are reversed due to the situs inversus. The anterior muscles similarly possibly represent the divided large papillary muscle that exists in the normal heart in the right ventricle. The presence of the non-coronary aortic cusp anteriorly is due to the failure of torsion of the great vessels.

Lewis and Abbott²² attempted to explain the difference that exists between the heart reported by Holmes, without transposition of vessels, and the remaining hearts of this type of cor triloculare which had a complete transposition of great vessels by assuming that the latter hearts were developed upon a reversed ventricular loop producing the transposition of vessels, while the heart reported by Holmes was developed in the normal position. But it is not clear from their models how both variations would produce a septum on the right, as exists in all those hearts. A reversal of the ventricular loop would bring the septum and great vessels to the left, as described in the reported specimen. No evidence is described of a situs inversus of the ventricles in these hearts. It appears, then, that the

process they believed took place in the hearts with complete transposition of the vessels with displacement of the septum to the right, actually took place in this heart, producing a septum on the left and a corrected transposition of the great vessels. In the former hearts, and also in that reported by Holmes, the right-sided septum occurred with the ventricles in their normal position. With the exception of the heart reported by Holmes, transposition of vessels has always occurred with this type of functional cor triloculare. The transposed relationship is frequently present in true three-chambered hearts.

Thus, the major abnormalities that exist in this heart are accounted for, and the steps in their pathogenesis are explained on the basis of a transposition of vessels occurring with a situs inversus of the ventricles and a primary malposition of the interventricular septum.

The clinical manifestations of functional cor triloculare are similar to those of true three-chambered hearts. Both varieties are compatible with fairly long life. The maximum age of the 11 individuals in the cases mentioned above was 23 years, the minimum, 9 weeks. Cyanosis, although at times severe, was usually present only to a slight degree, indicating a relatively small amount of free admixture of venous and arterial blood in these hearts. Clubbing of the fingers was absent or slight. Murmurs were inconstant, and the physical signs were not characteristic. Suffocative attacks were a frequent occurrence. Cardiac decompensation was the usual terminal event.

SUMMARY

A case is presented of functional cor triloculare biatria in which the interventricular septum was displaced to the left, dividing a large right chamber containing both auriculoventricular orifices and the pulmonary artery from a small left chamber which gave rise to the aorta.

The clinical manifestations of this anomolous heart are presented and its pathogenesis is discussed.

NOTE: The author wishes to thank Dr. Max Lederer for his many valuable suggestions in the preparation of this paper, and Dr. Maude Abbott for her helpful criticism.

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DESCRIPTION OF PLATES

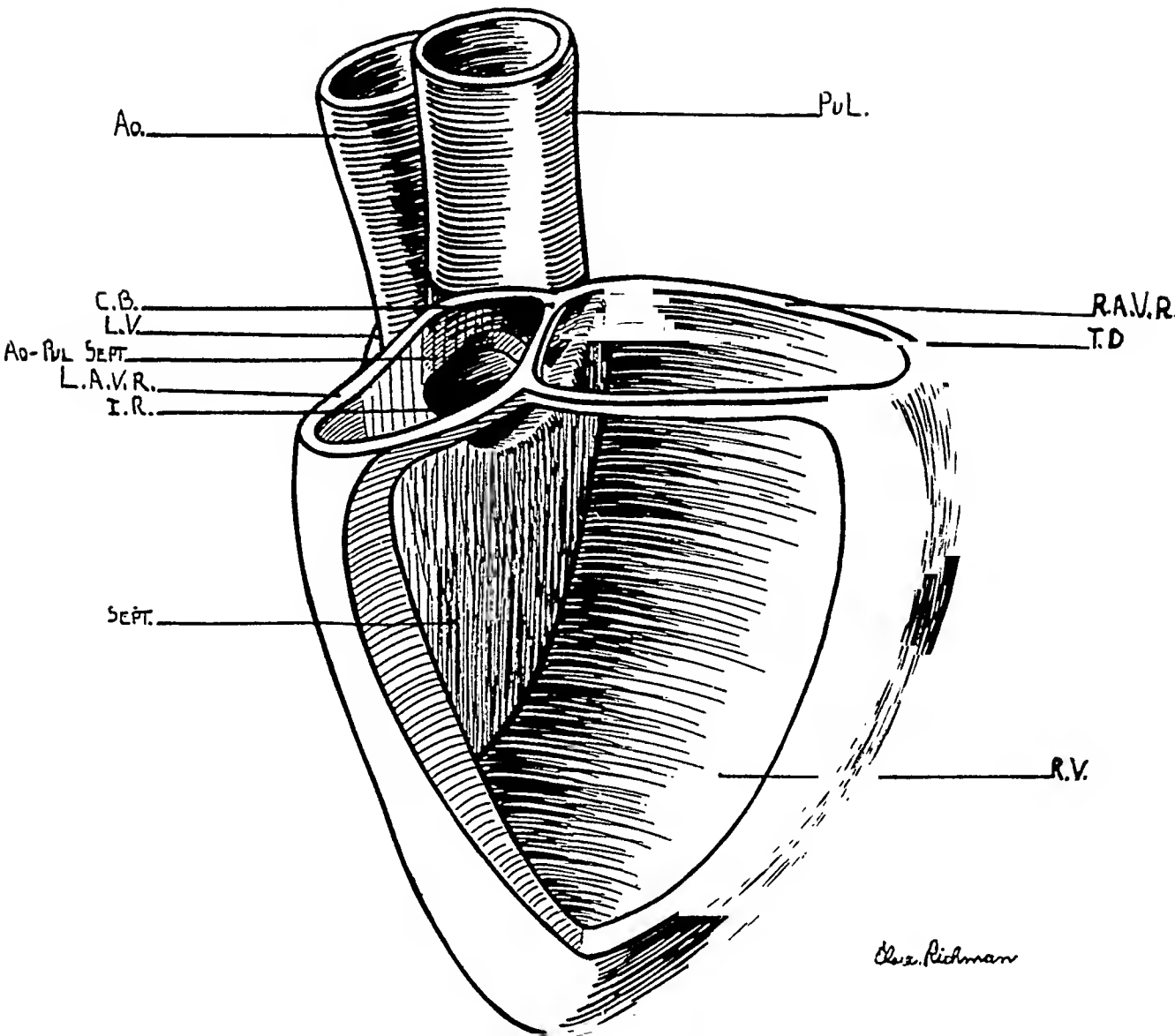
PLATE 115

FIG. 1. Diagrammatic drawing of heart viewed posteriorly, the auricles having been removed, leaving the auriculoventricular rings without the valves. A window is cut in the posterior wall of the right ventricle.

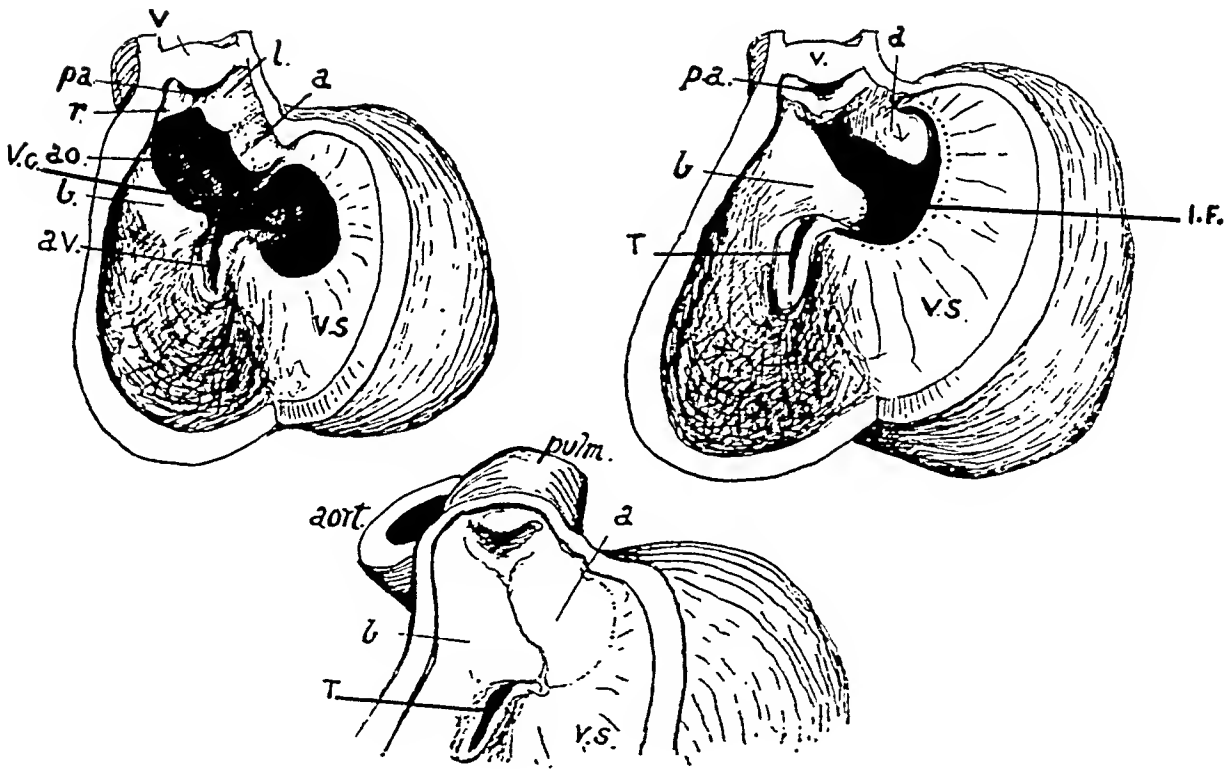
Ao. = aorta; Ao-Pul. Sept. = aortopulmonary septum; C. B. = common base of median cusps of both auriculoventricular orifices and free edges of interauricular septum; I. R. = interventricular foramen; L. A. V. R. = left auriculoventricular ring; L. V. = left ventricle; Pul. = pulmonary artery; R. A. V. R. = right auriculoventricular ring; R. V. = right ventricle; Sept. = septum in the ventricles; T. D. = triangular depression.

FIG. 2. Showing method of division of bulbar region and formation of aortic vestibule.

a = left bulbar ridge; b = right bulbar ridge; a v. = auriculoventricular orifice; T = right tricuspid orifice; V. S. = ventricular septum; V. C. = ventral endocardial cushion; I. F. = interventricular foramen (from Buchanan²³); p a = pulmonary artery; r = right bulbar cushion; l = left bulbar cushion; a o = aortic opening.



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PLATE 116

FIG. 3 A. Posterior view of heart with posterior wall elevated, exposing the interior of the right ventricle and both atria.

FIG. 3 B. Left anterolateral view of heart showing interior of left ventricle and aorta.



Kornblum

Cor Triloculare Biatra

AN IMPROVED TECHNIQUE FOR SILVER IMPREGNATION OF RETICULUM FIBERS*

HELENOR CAMPBELL WILDER

(From the Army Medical Museum, Washington, D. C.)

The argentation of reticulum fibers as an aid in the classification of tumors has been in use in this laboratory since 1924. Foot's^{1,2} technique has been the routine method employed; Laidlaw's³ method has been used occasionally. It has been previously reported (Wilder⁴) that glia cells in paraffin sections of formalin-fixed tissues may be impregnated, rapidly and without the use of heat, by sensitization with uranium nitrate prior to the exposure to ammoniacal silver (Foot's silver diammino hydroxid). The method presented in this paper is based on Foot's reticulum stain and the sensitization method for glia. It is very rapid and stains the finest reticulum fibers with great precision, collagen appearing rose-colored as in Foot's stain. It is applicable to formalin or Zenker-fixed tissues and to paraffin, celloidin and frozen sections. The problem of preventing sections from washing off the slides in ammoniacal silver has been solved in some measure by reduced exposure to this solution and the elimination of heat. However, the first loosening of the section occurs in the potassium permanganate solution and, even with this new method, occasional sections affixed with glycerine-albumin do not adhere throughout the subsequent procedures. In the staining of these difficult sections it has been found that substitution of phosphomolybdic acid for potassium permanganate in the pre-treatment gives satisfactory results and does not loosen the sections. We use hydrobromic acid rather than oxalic acid following either potassium permanganate or phosphomolybdic acid, although this step may be entirely omitted with but slight loss of definition after phosphomolybdic acid. Both methods are given in the following outline because the phosphomolybdic acid variation has not been in use a sufficient length of time to afford an accurate comparison with the results obtained by potassium permanganate, particularly on celloidin and frozen sections.

* Received for publication April 8, 1935.

TECHNIQUE

Fixation: Fix tissues in 10 per cent formalin, acetic-Zenker or formol-Zenker.

Embedding Tissues, Cutting and Mounting Sections: Embed in paraffin or celloidin, or cut frozen sections. Paraffin sections are mounted according to the routine method with Mayer's glycerin-albumin and are run through xylol, graded alcohols and distilled water before staining. Celloidin sections may vary in thickness from 4 to 30 microns. The thick sections give a better idea of the density of the fibers in some tumors. They are stained in dishes before mounting. Frozen sections may be stained in dishes before mounting, or mounted on slides and attached with thin celloidin before staining.

Pretreatment: Place the sections in 0.25 per cent potassium permanganate or in 10 per cent phosphomolybdic acid for 1 minute. Rinse in distilled water and place in hydrobromic acid (Merck's concentrated, 34 per cent, 1 part; distilled water, 3 parts) for 1 minute. Hydrobromic acid may be omitted following the use of phosphomolybdic acid.

Sensitization: Wash in tap water, then in distilled water and dip in 1 per cent uranium nitrate (sodium free) for 5 seconds or less.

Impregnation: Wash in distilled water 10 to 20 seconds and place in silver diammino hydroxid (Foot ⁵) for 1 minute:

To 5 cc. of 10.2 per cent silver nitrate add ammonium hydroxid drop by drop until the precipitate which forms is dissolved. Add 5 cc. of 3.1 per cent sodium hydroxid and just dissolve the resulting precipitate with a few drops of ammonium hydroxid. Make the solution up to 50 cc. with distilled water.

Reduction: Dip quickly in 95 per cent alcohol and reduce for 1 minute in the following solution:

Distilled water, 50 cc.; 40 per cent neutral formalin (neutralized with magnesium carbonate), 0.5 cc.; 1 per cent uranium nitrate, 1.5 cc.

Toning: Wash in distilled water and place in 1:500 gold chloride (Merck's reagent) 1 minute. Rinse in distilled water. Place in 5 per cent sodium thiosulphate (hyposulphite) 1 to 2 minutes.

Counterstaining and Mounting: Wash in tap water; counterstain, if desired, with hematoxylin and Van Gieson, or hematoxylin and

eosin; dehydrate in alcohol. Clear in xylol and mount in balsam. The use of ammonia must be avoided in blueing sections after hematoxylin as it dissolves the silver.

The use of distilled water and clean glassware for all solutions is essential. All the solutions may be used repeatedly and kept in Coplin jars for several days. The solutions keep without disintegrating in amber glass-stoppered bottles for an indefinite time.

SUMMARY

Sensitization of sections with uranium nitrate before silver impregnation has reduced the time required for staining reticulum fibers to 10 minutes and has eliminated the necessity of heating the ammoniacal silver. In this way the difficulty of staining sections which are inclined to wash off the slides has been greatly lessened. The substitution of phosphomolybdic acid for potassium permanganate is recommended for such sections as still persist in coming off the slides.

In our experience hydrobromic acid substituted for oxalic acid in the pretreatment, and uranium nitrate substituted for the sodium carbonate of Foot's reducing solution, have improved the staining of argyrophil fibers.

The method presented here is now in routine use in this laboratory. It has proved a time saver and has given even more satisfactory results than the older methods.

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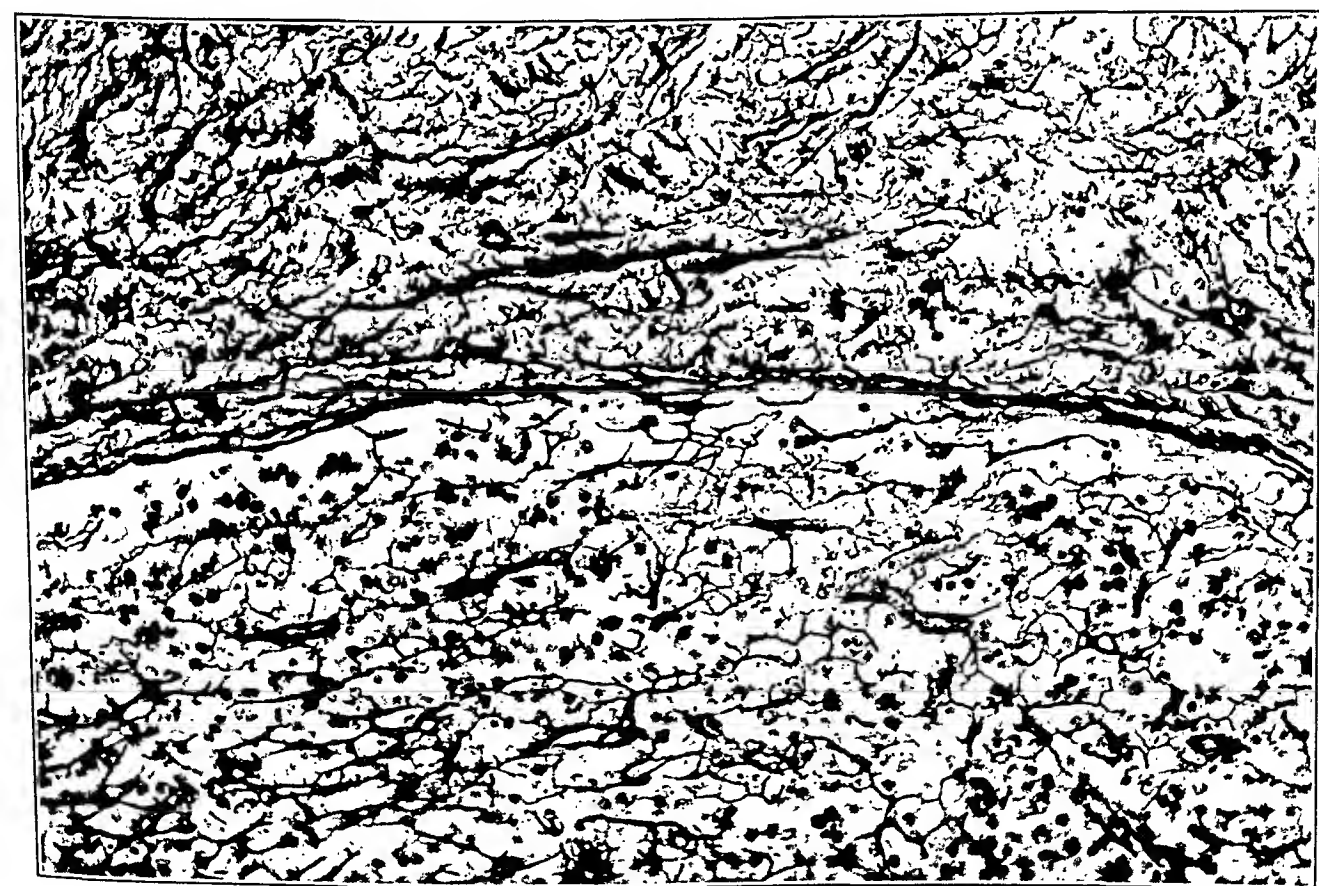
DESCRIPTION OF PLATE

PLATE 117

- FIG. 1. Silver impregnation of reticulum fibers in a lymph node from a case of reticulum cell leukosarcoma. The slide was stained by the uranium nitrate sensitization method following potassium permanganate and hydrobromic acid. A.M.M. Neg. No. 63006. $\times 215$.
- FIG. 2. A slide from the same block stained by the uranium nitrate sensitization method following phosphomolybdic acid, without hydrobromic acid. A.M.M. Neg. No. 63006. $\times 215$.



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SCIENTIFIC PROCEEDINGS OF THE
THIRTY-FIFTH ANNUAL MEETING
OF THE
AMERICAN ASSOCIATION OF PATHOLOGISTS AND
BACTERIOLOGISTS

HELD AT CORNELL UNIVERSITY
MEDICAL COLLEGE,
NEW YORK CITY,
APRIL 18 AND 19, 1935

THE AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

ABSTRACT OF BUSINESS SESSION.

President BOYD in the Chair

The Secretary presented the nomination of the Council for officers as follows:

<i>President</i>	S. BURT WOLBACH
<i>Vice-President</i>	N. CHANDLER FOOT
<i>Treasurer</i>	F. B. MALLORY
<i>Secretary</i>	HOWARD T. KARSNER
<i>Incoming Member of Council</i>	C. V. WELLER
<i>Assistant Treasurer</i>	FREDERIC PARKER, JR.
<i>Assistant Secretary</i>	ALAN R. MORITZ

Voted unanimously to elect those nominated.

Voted to elect the following new members:

William Antopol	Willard S. Hastings
Francis Bayless	Hardy A. Kemp
Allan W. Blair	Gustavus H. Klinck, Jr.
Alfred Blumberg	Enrique Koppisch
Paul Brindley	Leila C. Knox
Osborne A. Brines	Marshall M. Lieber
Edward M. Butt	James B. McNaught
Jefferson H. Clark	John E. McWhorter
Marion C. Corrigan	Perry J. Melnick
Alvin J. Cox, Jr.	W. J. Nungester
Dominic A. DeSanto	Leland W. Parr
Claude E. Dolman	Coleman B. Rabin
Joseph C. Ehrlich	Raymond S. Rosedale
Wiley D. Forbus	Samuel H. Rosen
Thomas Francis, Jr.	Sol R. Rosenthal
William Freeman	Hollis K. Russell
Harold Gordon	Samuel Sanes

Gordon H. Scott
Richard E. Shope
Douglas H. Sprunt

William S. Stanbury
Ernst Witebsky
Krikor Yardumian

It was also voted to reinstate Drs. Donald T. Fraser, DeWayne G. Richey and J. H. Pratt.

Voted to accept with regret the resignations of Drs. B. L. Arms, L. R. Jones, E. O. Jordan, R. H. Major and G. F. Ruediger.

Voted to record with deep regret the deaths of Drs. James Coupal, N. C. Davis, F. S. Jones, Ernest Scott, Theobald Smith, and William H. Welch.

Voted that the Symposium next year be on the subject of Inflammation.

Voted to request Dr. Arnold R. Rich as referee.

Voted to accept the invitation of Drs. F. B. Mallory and Frederic Parker, Jr., to meet in Boston April 9 and 10, 1936.

The Secretary pointed out that through the death of Dr. Theobald Smith a recipient of the Gold Headed Cane of the Association should be selected. It was unanimously voted to select Dr. Frank Burr Mallory for this honor.

Voted to elect Dr. Frederick P. Gay as representative of this Association on the Committee of Type Culture Collection.

AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

A VIRUS DISEASE OF OWLS. R. G. Green, Minneapolis, Minn.

Abstract. From a great horned owl (*Bubo virginianus*) found dead in the wild, a disease was experimentally transmitted to a second great horned owl and to a screech owl (*Otus asio*). Attempts to transmit the disease to a great gray owl (*Scoliaptex nebulosa*), pigeons and guinea pigs met with failure. The liver and spleen of the owl dying of the natural infection were studded with fine abscesses. Microscopic examination of the organs showed the liver to contain many microscopic abscesses. In the peripheral zone of the abscesses were many intranuclear inclusion bodies involving principally hepatic cells and occasionally an endothelial cell. Similar inclusion bodies were found in the livers of both owls experimentally infected. The distinctive nature of the intranuclear inclusions leaves no doubt that the infection is to be classed as a virus disease. The studies were carried out with Dr. J. E. Shillinger.

Discussion

(Dr. Francis G. Blake, New Haven.) Have you any indication as to the natural method of transmission of the virus?

(Dr. Thomas M. Rivers, New York City.) I should like to suggest, inasmuch as there is a virus disease of parakeets in Brazil exactly like that described by Dr. Green, that he attempt to transmit it to parakeets. Might you not find them a susceptible host?

(Dr. Green, closing.) Unfortunately as this virus was lost we were unable to carry out extensive studies on transmission, and we have no indication as yet as to how the disease is transmitted.

I appreciate Dr. Rivers' suggestion. We are carrying out extensive studies of wild life, and owls are continuously under observation. We have maintained a stock of horned owls now for 2 years, hoping to meet this virus again.

A FILTRABLE VIRUS FROM WHITE MICE. Erich Traub (by invitation), Princeton, N. J.

Abstract. If mice from our apparently healthy breeding colony are given an intracerebral inoculation of sterile bouillon a small proportion die after showing characteristic symptoms. Death is due to a filtrable virus which can be transmitted in series by intracerebral inoculation in mice and guinea pigs. The latter can also be infected by material injected subcutaneously or intranasally and are the best animals to use in detecting the virus. Rats inoculated intracerebrally develop the disease.

The outstanding pathological changes in experimentally infected mice are a marked meningitis and infiltration of the chorioid plexus with mononuclear cells, mostly lymphocytes. The virus is present in the blood and urine of diseased mice as well as in the central nervous system.

The pathological changes in guinea pigs are similar to those in mice, except that cellular infiltration of the meninges and chorioid plexus is much less marked than in mice. Acidophilic intranuclear inclusions were found in guinea pigs in meningeal cells, in mononuclear cells present along the meninges, in adventitial cells of meningeal vessels, in glia cells near the pia mater and, very rarely, in epithelial cells of the chorioid plexus. Pneumonia of the virus type is frequent in guinea pigs inoculated intranasally, intracerebrally, or subcutaneously with virus.

Cross-neutralization experiments show that our virus is serologically identical with the virus of lymphocytic choriomeningitis described by Armstrong (*Pub. Health Rep.*, 1934, 49, 1019). It has further been found that the blood serum of the man who takes care of our breeding mice neutralizes the virus recovered from the stock.

Discussion

(Dr. Thomas M. Rivers, New York City.) I think Dr. Traub's virus is of interest for two reasons: first, individuals who are working with viruses in mice might mistake this new virus for the active agents with which they are working. Second, Dr. Traub's virus is pathogenic for man. Dr. Scott and I saw two men who had the clinical picture of meningitis — stiff neck, fever, Kernig sign, and marked increase of cells in the spinal fluid, practically all of which were mononuclears. From the spinal fluid of these two patients we obtained a virus identical with that of Dr. Traub. The virus is not in our stock mice. Furthermore, the patients had no neutralizing antibodies for this virus at the onset of illness, but developed them during convalescence.

(Dr. Ralph D. Lillie, Washington.) The pathological response in Dr. Traub's mice apparently differs in some respects from that studied by Armstrong and myself in Washington in that we have seen less tendency to invasion of the central nervous system and the reaction appears to me to have been less marked in the choroid plexi than would be indicated from Dr. Traub's description. The meningeal reaction has been very similar in nature, however.

(Dr. Maurice Brodie, New York City.) I should like to ask Dr. Traub what concentration of the emulsion he used in these mice, because we have picked up in mice a spontaneous virus which seems to be a little different from his; that is, the incubation period is 2 to 4 days when we use a 10 per cent suspension. If we use weaker dilutions, 1:100 or 1:150 suspensions, the incubation period becomes longer. The disease is rather more acute; convulsions occur spontaneously and there is much more tremor. The pathology is a little different, inasmuch as meningeal involvement is not as marked, and there is but little choroid involvement, more hyperemia, and focal areas of necrosis in the posterior horns are found. I wonder whether the greater reaction we get can be due to greater concentration and whether we are dealing with the same thing. We have not succeeded in transferring it to guinea pigs, but we have given it to rabbits.

(Dr. Traub, closing.) In reply to Dr. Lillie, the necrosis of nerve cells does not occur in all mice and the number of necrotic cells is fairly small, but we considered the necrosis was definite because we got neuronophagia and all the features which are essentially connected with necrosis.

I should like to answer Dr. Brodie that it does not make much difference what the concentration of virus is that we inoculate into mice. The incubation period is not shorter than 5 days.

THE VIRUS OF LYMPHOGRANULOMA INGUINALE. Rigney D'Aunoy, Emmerich von Haam and (by invitation) Louis Lichtenstein, New Orleans, La.

Abstract. Although lymphogranuloma inguinale appears to be a rather common disease in the United States and numerous case reports have been published from various parts of this country, comparatively little has been done by American authors in systematically studying its causal virus. In the course of our clinical study of this disease in its various manifestations in New Orleans, 160 cases were observed over a period of 6 months. From 7 of these cases the filterable virus etiologically associated with the disease was isolated and kept viable and infectious through repeated animal passage. The physical and biological characteristics of these local virus strains were studied and compared with similar findings of European investigators. This was accomplished by means of: (1) animal passage of the virus through homologous and heterologous species of experimental animals, using various routes of inoculation; (2) consideration of the antigenic value of fresh and desiccated organ emulsions of infected animals for the diagnostic Frei reaction; (3) cultivation of the virus; (4) animal protection experiments, and (5) histopathological studies.

Discussion

(Dr. Arthur William Grace, New York City.) I have enjoyed the presentation of this paper very much. Dr. von Haam has covered a great deal of work in a comparatively short time and the results he has found have been very much in line with what we have found in the work on lymphogranuloma inguinale. There is one point on which we differ: he spoke of the autosterilization of the virus. We have not detected that at all. We have now passed our strain in 12 months through 70 to 75 generations of white mice and have not detected any trace of autosterilization whatever. The virus is just as strong now as during any passage en route. Whether we are dealing with a stronger virus I do not know; I rather feel it is, because our results from the beginning showed a shorter period of incubation in mice than has been reported by any other worker. Another point that we have been working on here is the use of the infected brain as an antigen. We have obtained no non-specific reactions, and the material obtained from the mouse brain has produced as strong a Frei reaction as can be obtained with the most potent human lymphogranulomatous pus. The lack of good Frei antigen has hampered the diagnosis of the disease for a long time, but now that the disease can be transmitted so readily to mice and the infected mice brains do not contain a suspicion of any other venereal disease, mouse brain antigen is very suitable for distribution. Lederle has taken the mouse strain of virus we are using and is now putting up the material commercially, so that I suspect in a short time lymphogranuloma inguinale mouse brain antigen will be available in an unlimited supply for the diagnosis of the disease, and then we shall see what a large number of cases there must be scattered throughout the country. We shall probably find there are more rectal than inguinal cases, because the rectal cases are with us all the time. In the inguinal cases the patients get better and pass out of sight, and I think the rectal surgeons will find the use of lymphogranulomatous mouse brain very valuable for the diagnosis of a number of obscure cases of benign inflammatory rectal disease. It is due to the efforts of Dr. von Haam and his co-workers and the people in France and Scandinavia that we have been able to replace the very unesthetic human lymphogranu-

lomatous antigen by the animal antigens which are just as specific and always available in large quantity.

(Dr. Maurice Brodie, New York City.) I should like to ask whether or not all of Dr. von Haam's animals came down with symptoms, or whether a good many did not, and whether he transferred the virus after 2 to 4 weeks in the manner Levaditi has. We have tried 6 or 7 strains by that method but have not had the success which he had. In 2 we got the transfer to mice, and 1 to guinea pigs, and none of the animals came down with symptoms. Two to 4 weeks after the injection some of the mice would die, and so we tried transferring it at 2 week intervals and tested the material in patients, and we found the reactions in the skin got weaker with successive transfers until it lost all its strength, usually in 3 or 4 passages, and so I wondered what kind of reaction he had in the majority of his animals.

(Dr. Enrique E. Ecker, Cleveland.) I should like to ask Dr. von Haam if any attempts have been made to use the culture instead of the usual Frei antigen, and whether the culture or brain emulsion will give skin reaction test in high dilutions. We got a reaction with the Frei antigen in 1 case using a dilution of 1:20,000. However, reactions occur commonly with dilutions of 1:5000. A positive test has been obtained as long as 39 years after the infection.

(Dr. Morris I. Rakieta, New Haven.) I should like to ask Dr. von Haam about the cloudiness in the medium. Does he interpret that as an actual growth, a proliferation of the virus in an artificial medium?

(Dr. William Boyd, Winnipeg.) How many cases of the disease due to this virus have been reported in Canada?

(Miss Suskind, New York City.) I should like to ask Dr. von Haam, in relation to the cultural activity of the virus, whether he has been able to stain the fluid and demonstrate what might be described as filterable virus bodies.

(Dr. von Haam, closing.) In regard to autosterilization, we have observed it twice in our 7 strains. Two of our strains died out through continual passage through mice. I believe Dr. Grace has an especially strong virus strain, because of the incubation time and because he has successfully transmitted it so long.

As to the distribution of the disease in Canada, there is only one report in a French Canadian journal which unfortunately was not available to me. That reported 3 cases in the vicinity of Quebec.

I am unable to answer the question as to the dilution of the virus and of the brain emulsion as we did not work with this problem. The literature reports usually the virus cannot be diluted very much and negative results in a dilution of as high as 1:10,000 are reported.

In regard to Dr. Brodie's remarks as to symptoms, we keep the virus now going by routinely transmitting the virus every 2 weeks, and most of our mice do not show symptoms by this time. The incubation time in our strains for the appearance of symptoms is 3 to 4 weeks, but characteristic histological changes can be found in mice as early as 5 to 6 days after the inoculation.

In reply to Miss Suskind's remarks about staining the virus, we did not stain the virus. However, Tamura stained the fluid with virus stain, and he found bodies which he believes to be virus.

I do not know that the cloudiness actually represents the virus, but it does not appear in the control animals. The cloudiness can be transmitted from tube to tube and, as Tamura has shown, the virus can be centrifuged with about 6000 revolutions per minute down in the deeper layers.

THE PRESENT STATUS OF THE ANTIGENIC ANALYSIS OF THE ELEMENTARY BODIES OF VACCINIA. James Craigie (by invitation), Toronto, Canada.

Abstract. It has been reported that two types of reaction may occur when suitably prepared extracts of vaccinia-infected tissue are incubated with rabbit antivaccinia serum (*Brit. J. Exper. Path.*, 1932, 13, 259). A precipitin reaction takes place with "free" antigen in the extract and, in addition, any elementary bodies which may be present are agglutinated. More recently it has been shown that rabbit antivaccinia serum contains two types of agglutinin for the elementary bodies of vaccinia (*Brit. J. Exper. Path.*, 1934, 15, 390). The corresponding agglutinogens differ markedly in their stability. The L or labile antigen is inactivated by heat at 56° C., by excess of formalin, by the photodynamic effect of methylene blue, and by other manipulations which render the elementary bodies non-infective. The S or stable antigen, on the other hand, withstands a temperature of 100° C. This paper presents further observations on these antigens.

It may be shown by means of suitable precipitin tests on the wash fluids that in a freshly prepared and washed elementary body suspension all the antigen is fixed to the surface of the elementary bodies. On storage, however, particularly if distilled water saturated with ether is employed as the suspending fluid, a portion of the antigens becomes dissociated from the elementary bodies. These dissociated antigens are demonstrable by subjecting the suspending fluid, after removal of the elementary bodies, to precipitin tests. The use of serums containing either L or S antibody alone has shown that both the corresponding antigens dissociate. Dissociated L antigen is as labile as L antigen fixed to the elementary bodies. Repeated but decreasing yields of "free" antigen may be obtained and the elementary bodies remain agglutinable and infective. However, the appearance of fine particles in the suspension, and a decrease in infectivity, suggest the possibility of disintegration of some of the elementary bodies of lesser resistance.

Pure L and S precipitin serums, controlled by appropriate tests with elementary bodies and antigen dissociated from them, have been used to determine the nature of the antigens found in Seitz filtrates of vaccinia-infected skin. Such filtrates, free from elementary bodies, contain both L and S antigen. The conditions under which these filtrates are prepared make it seem highly probable that the antigens exist in the "free" state in vaccinia-infected skin. Both L and S antigens have been found in the vaccinia-infected skin of the calf and guinea pig, as well as in that of the rabbit.

It has been suggested that the "antigens" involved in the *in vitro* reactions of some viruses are of extrinsic origin, arising from host substances as a result of the infective process. No observations have been recorded which are inconsistent with the view that the L and S antigens of vaccinia are intrinsic products of the elementary bodies, analogous to bacterial antigens, which may dissociate *in vivo* as well as *in vitro*. The comparatively large quantities of L and S antigen which seem to be present in the "free" state in vaccinia-infected skin, taken in combination with other observations, would seem to suggest that vaccine virus particles may vary greatly in their resistance to disintegration. Elementary body suspensions prepared by the usual methods may possibly represent only the more stable forms of the virus with spore-like qualities of high resistance to an adverse environment. Actively proliferating intracellular virus, not neces-

sarily differing in morphology from elementary bodies, may be much less viable if separated from living cells.

The possibility that vaccine virus particles of varying viability when separated from the cell may exist in living tissue, but that only the more resistant forms can be manipulated *in vitro*, should be borne in mind in interpreting failures to demonstrate inactivation of the virus by immune serum *in vitro*.

Discussion

(Dr. Frederick P. Gay, New York City.) I should like to ask which of these two antigens gave rise to immunity, or whether they both did.

(Dr. Claus W. Jungeblut, New York City.) I did not quite hear what Dr. Craigie said about the neutralization experiments, but I would like to know which of the two serums, the S or the L type, was more active in neutralizing the virus *in vitro*.

(Dr. Craigie, closing.) I am afraid that I have been asked two questions which I cannot answer. I do not know which of the two types of antibody is more active in neutralizing the virus or whether either of them is active in this respect. As regards immunity, I think that I can say this — there is no evidence which would indicate that the S antigen and corresponding antibody play any significant part in immunity. The L antigen may be involved, but I am not certain. It is true that vaccinia elementary bodies inactivated without loss of the L antigen appear to produce a much greater degree of immunity than elementary bodies inactive with concomitant loss of this antigen. This, however, does not necessarily mean that the L antigen is involved in immunity. There may be some other labile antigen which we cannot demonstrate *in vitro*.

RESPONSE OF RABBITS TO FORMOLIZED WASHED ELEMENTARY BODIES OF VACCINIA AND TO VIRUS-FREE FILTRATES OF DERMAL VACCINE VIRUS. Robert F. Parker (by invitation), New York City.

Abstract. It has been shown by Craigie that injections in rabbits of inactive elementary bodies of vaccinia cause the appearance of agglutinins, precipitins, and complement-fixing antibodies. Washed suspensions of elementary bodies were prepared by Craigie's technic, which were almost entirely free of other particulate material. The virus was inactivated by means of 0.3 per cent formaldehyde, large amounts being tested for the presence of active virus by serial testicular passage in rabbits. Extracts of dermal virus containing the soluble antigens of vaccinia were obtained and freed of virus by passage through collodion membranes. These were similarly tested for the presence of active virus.

Rabbits kept under isolation precautions were injected twice weekly for 6 weeks with increasing amounts of these materials, a total of 18 cc. being inoculated. Serum was taken and the rabbits were tested for immunity by means of dermal, intradermal and testicular inoculations of virus.

After immunization, agglutinins for elementary bodies and precipitins against the soluble antigens were present in moderately high titer, while virus-neutralizing antibodies were present in small amount. The rabbits were moderately or completely refractory to infection with a weak culture virus, but only partially immune to a stronger testicular strain.

A striking phenomenon was noted, *i.e.* animals immunized with inactive elementary bodies, and almost entirely refractory to infection with culture virus, were still moderately susceptible to a later inoculation of potent testicular virus.

Discussion

(Dr. James Craigie, Toronto.) Dr. Parker's paper raises some interesting points. I might say in the first place that his results are in agreement with those obtained by us in our earlier attempts to produce immunity with inactivated vaccinia elementary bodies. The observations made during the course of this preliminary work were one of the factors in determining a search for a labile type of antigen in the elementary bodies of vaccinia. I should emphasize that Dr. Parker in his formalized washed elementary bodies has used an amount of formalin which in my experience will completely inactivate the L antigen as well as abolish the infectivity of the elementary bodies. More recently I have been attempting to inactivate the elementary bodies of vaccinia with the least possible amount of formalin and this has been facilitated by the use of pure L sera. If one succeeds in inactivating the elementary bodies, leaving the labile antigen more or less intact, then one can produce a much more definite degree of resistance to skin inoculation of vaccine virus. This resistance may be measured in two ways, and the results may differ somewhat according to the method of the test, *i.e.* whether skin scarification or intradermal injection is employed. One manifestation is a definite resistance to inoculation of the virus, and the other is a very definite acceleration of the reaction. One may in rabbits produce quite marked resistance to inoculation with inactivated elementary bodies, but this immunity does not compare in its duration to immunity developed after inoculation of living elementary bodies. I think that the present interest of this type of investigation lies not so much in the attempt to produce immunity by inactivated vaccinia virus, but in the investigation of the mechanism of immunity to this virus.

(Dr. Harry S. Eagle, Philadelphia.) I think it is interesting that an antigen was found that would pass through a collodion membrane. It indicates that particles of small molecular size may be antigenic. I should like to ask Dr. Parker if he has any data on the permeability of the filter he used.

(Dr. Parker, closing.) In reply to Dr. Eagle, the membranes used were prepared by Bauer in the Yellow Fever Laboratory and the average diameter of the pores is 103 millimicra.

DEVELOPMENT OF IMMUNITY TO FOX ENCEPHALITIS. R. G. Green, Minneapolis, Minn.

Abstract. Serum of animals recovered from fox encephalitis contains antiviral and this can be increased by hyperimmunization. The maximum antiviral content is developed after more than 1 year's continuous injection of virus. The injection of serum-virus mixtures into normal foxes leads to death of the more susceptible animals after 30 days, when the serum has been eliminated. Development of acquired immunity to fox encephalitis seems to require several weeks for the most susceptible individuals. Recovery from fox encephalitis appears not to depend upon acquired immunity, but upon the extent of natural immunity at the onset of the disease. The studies were carried out with Dr. J. E. Shillinger.

Discussion

(Dr. E. Watson, Ottawa.) I should like to ask Dr. Green if he has done any cross-immunization tests with fox encephalitis and canine distemper virus and, if so, has he found that one has any neutralizing effect on the other?

We have done a few experiments along those lines, and there is a tentative suggestion in them that there is some protection offered by canine distemper virus against fox encephalitis virus, and *vice versa*.

(Dr. Claus W. Jungeblut, New York City.) I should like to ask Dr. Green if he has formulated any ideas about the mechanism of the natural immunity he spoke of in his paper. Is it acquired by previous infection?

(Dr. Green, closing.) In reply to Dr. Watson's question, I should like to state that our extensive investigations have been confined largely to a particular strain of virus, the Fromm strain, and we have been unable to discover any relation whatsoever with the canine distemper virus.

Dr. Jungeblut's question is one with which we have concerned ourselves. In our experimental ranch we maintained strict quarantine for a number of years. The foxes on the ranch were principally red fox pups dug out of their dens in the wild and transported by litters to the ranch. We are quite positive that the immunity shown by foxes from this group was not acquired by contact with the virus. We have studied the breeding records of fox groups being put into an epidemic area each fall. It was a very striking observation that each year the progeny of certain pairs of foxes would always die of fox encephalitis, while the progeny of other pairs never died of the disease, although all were exposed together. This definitely appears to be evidence of inherited natural immunity.

STUDIES ON THE MECHANISM OF IMMUNITY IN CERTAIN VIRUS DISEASES. Albert B. Sabin (by invitation), New York City.

Abstract. Since the protective substance in immune, antiviral serum is capable of exerting its effect *in vitro* in a simple system consisting of minced susceptible tissue, immune serum and virus, the rôle and fate of each of the constituents in the consummation of the immune process were analyzed. With the aid of an ultracentrifuge (14,000 r.p.m.), which rendered possible the complete sedimentation and quantitative recovery of vaccinia, pseudorabies and B virus, it was found that the protective substance in antiviral serums neither combined with nor inactivated these viruses even after prolonged periods of incubation. In cultures containing susceptible tissue, immune serum and virus, the protective effect was exerted without any demonstrable direct effect upon the virus; the protective substance apparently acted upon the tissue by rendering it refractory to "infection," and unsuitable for the multiplication of the virus. By treating normal susceptible tissue with antiviral serum and then separating the tissue from the serum, it was possible to show that it had been rendered refractory to infection even though it was surrounded by and had "fixed" active virus. It was also found that the protective substance is fixed by the tissue and that both the fixation and the effect are reversible by washing. Experiments with leukocytes revealed that while they take up or fix virus, they become highly infectious thereby and thus play no part in preventing infection; no opsonic effect of immune serum on virus was demonstrable.

The varying protective power of antiviral serum *in vivo* in different tissues of the same species and in the same tissues of different species was studied: it was found that this phenomenon could be accounted for neither on the basis of any direct action of the immune serum upon the virus *in vitro* or *in vivo*, nor on the basis of the varying ability of the tissues to neutralize small amounts of virus. It appeared rather that different tissues require different amounts of protective substance for protection against the same amount of virus and that certain

other, as yet obscure, factors are involved. These studies suggest that the virus itself may not be the direct antigenic stimulus, but that some substance upon which it acts and which becomes antigenic when liberated from infected cells may be the factor responsible for the formation of the immune protective substance.

Discussion

(Dr. R. G. Green, Minneapolis.) I shall look forward with a great deal of pleasure to seeing the details of this paper, as one of the conclusions does not appear to correspond with our experience. In one of our experiments 25 foxes were given an injection of serum to produce a passive immunity. Of the first 10 foxes inoculated with virus 10 days later, 8 died with typical encephalitis. The same amount of serum would have given protection for 30 days if the serum had been mixed with the virus before injection. This would make it appear that the effect of the serum was on the virus rather than on the tissue. We also have seen the variation of immunity apparently dependent upon the tissues involved. In our preliminary investigations on virus neutralization all inoculations were made by cisterna puncture. In these experimental animals we observed no delayed infections. Among some 2000 ranch foxes inoculated with similar mixtures by intramuscular injection, the disease regularly appeared some 30 days after inoculation as delayed infections. Following this the delayed infections were observed in experimental animals when the serum-virus mixture was injected intramuscularly.

(Dr. Joseph D. Aronson, Philadelphia.) I should like to ask what tissues are especially susceptible to the virus, whether you can produce selective immunity in such tissues, and lastly, whether the serum can be removed from the tissues so as to again make the tissue susceptible to infection with the virus.

(Dr. L. Dienes, Boston.) In connection with the observation which I reported yesterday, I should like to mention that in guinea pigs the development of immunity response after infection with vaccinia virus is about the same as after injection of eggwhite. If a large area of the skin is infected, antibodies appear in the blood first after the 12th day. However, the skin lesions begin to heal on the 5th day. At about the same time the non-infected part of the skin begins to give slight hypersensitive reactions. These reactions are rather strong on the 8th day. These skin reactions correspond to the tuberculin reaction and to the slight skin reaction which we observed between the 4th and 7th days after eggwhite injection. They are characterized by delayed appearance and a mainly mononuclear infiltration of the tissues. This type of skin sensitiveness corresponds to the active immunization of the tissues, and circulating antibodies never produce a similar condition. In guinea pigs the infection of the skin heals in the active stage of the immunization process before the appearance of antibodies in the circulation. We do not know the relation of antibodies to this condition; it certainly cannot be reproduced by passive immunization. The study of this condition may be as necessary for the understanding of healing and immunity as the study of antibodies.

(Dr. Francis G. Blake, New Haven.) It would appear to me that the conclusions from these experiments are consistent with some of the observed phenomena met in disease. I shall cite only two: first, the observed fact that in the prophylactic use of convalescent serum in measles the serum must be used before the virus enters the blood, if prevention is to be obtained; second, the ob-

served fact that in herpes febrilis the disease may frequently recur in an individual in spite of the fact that he has neutralizing antibodies in his blood. I should like to ask Dr. Sabin if these two observed facts, which are illustrative of others, seem to him consistent with his conception.

(Dr. Sabin, closing.) In reply to Dr. Green, I must say that I have made a careful study of many of his interesting experiments with the virus of fox encephalitis and found them strikingly in accord with the conception that the protective action of antiviral serum is not the result of any direct action on the virus. In one particular type of experiment, in which Dr. Green injected mixtures of serum and virus subcutaneously, the animals appeared to be completely protected for a long period (a month or more), and then suddenly would develop the disease. During this entire period apparently nothing happened to damage the virus, and when the passive immunity conferred by the serum had worn off the animal developed the typical disease. Somewhat similar experiences have been encountered in dog distemper work when immune serum and virus were used for immunization. The fact that Dr. Green observed no delayed infection when he used the cisternal route may mean that the virus does not survive as long there as in the subcutaneous tissue. The spontaneous deterioration of viruses which may vary in different tissues may play an important part in determining the ultimate infectivity of a serum-virus mixture.

In response to Dr. Aronson, the susceptibility of different tissues probably varies with individual viruses. I cannot answer the second question, but the third one regarding the possibility of removing the serum from immune tissue and thus rendering it more susceptible to infection can be answered in the affirmative. Rivers and co-workers have shown that the excised cornea from a rabbit immune to vaccinia may occasionally be deprived of its resistance to infection *in vitro* by washing, and Andrewes found that testes from actively immune herpes and virus III rabbits were rendered highly susceptible to *in vitro* infection by washing. In my own work it was shown that while normal tissue could be rendered refractory to infection by exposure to immune serum, it would lose this refractory state upon repeated washing.

The observations on the prophylactic effect of measles convalescent serum chiefly before the generalization of the virus and on the occurrence of herpes febrilis in individuals with antibodies for the virus in their blood, which Dr. Blake has mentioned, would not appear to be inconsistent with the conception that the antibodies in immune serum do not act directly upon the virus. In the case of measles, it is clear that the cells must be, and perhaps are, protected before the virus reaches them to avoid the systemic disease, while in the case of herpes febrilis the present conception explains at least why a virus survives and may be carried by an immune host in the presence of immune bodies, which do not "neutralize" it even when it is unprotected by a probable intracellular habitat.

EVIDENCE OF ACQUIRED IMMUNITY FROM PLANT VIRUS DISEASES. L. O. Kunkel (by invitation), Princeton, N. J.

Abstract. Tobacco seedlings regularly recover from, and are thereafter immune to, the disease caused by the tobacco-ringspot virus. Recovered plants, although indistinguishable from plants that have never had the disease, retain the ringspot virus indefinitely. Plants propagated from cuttings of recovered plants are likewise immune, but plants grown from seeds are susceptible.

More than fifty different strains of the tobacco-mosaic virus have been isolated. The several strains cause diseases that differ in severity, from some that are extremely mild to others that are lethal for young plants. Seedlings infected by a mild strain of virus become immune from severe strains. They are not, however, protected against tobacco ringspot, cucumber mosaic, or any plant virus disease unrelated to tobacco mosaic. The protection test furnishes an easy means of determining whether or not any new disease belongs in the tobacco mosaic group. Similar tests with the tobacco-ringspot virus and with viruses causing other diseases indicate that the immune reaction is specific. Results obtained from protection tests have been confirmed by serological studies.

Discussion

(Dr. Everett G. D. Murray, Montreal.) I should like to ask Dr. Kunkel if the susceptible plant is budded or grafted on to an immune or recovered plant whether there is any protection or resistance transferred to the bud or graft.

(Dr. Kunkel.) No, the bud or graft does not acquire immunity without an attack of the disease.

(Dr. James Ewing, New York City.) I should like to ask Dr. Kunkel to tell us a little more about the conditions of survival of the virus in plants that do not show the symptoms of the disease.

(Dr. Augustus B. Wadsworth, Albany.) I should like to know how long the virus persists in the transplants of the cuttings. I assume that it continues.

(Miss Suskind, New York City.) Before I ask my question I must plead ignorance of botanical technique. I would like to know, however, if it is possible to macerate recovered plant tissue and demonstrate tissue immune bodies, either by prophylactically treating well plants with that macerated plant extract and subsequently attempting to infect them, or by taking plants already infected and treating them with the macerated extract.

(Dr. Stuart Mudd, Philadelphia.) Is it possible to obtain passive immunity by transfer of sap or other plant juices?

(Miss Feig, New York City.) There appear to be about fifty different types of plant virus disease. How do you tell the different types apart?

(Dr. Arthur William Grace, New York City.) I should like to ask whether the seeds of plants that are non-immune come from plants that were previously infected, or whether they are seeds from other plants.

(Dr. Kunkel, closing.) Regarding the seeds, to answer the last question first, the viruses do not usually pass through the seeds. There are a few exceptional cases, like the virus disease of the bean, which is transmitted through the seeds, but most viruses are not transmitted through the seeds of plants. Plants grown from seeds of diseased plants are quite healthy.

Regarding how long the virus persists in cuttings, we have some cuttings that have been grown in successive generations for over a period of 4 years, and the virus is there just the same as when the plant first recovered.

In reply to Miss Feig, as to how we recognize these different strains, we recognize them by the symptoms they produce in the plants.

In reply to Miss Suskind, we have no evidence whatever of immune bodies. We know nothing of the mechanism by which the plants are protected. You must remember there is no blood stream to play with.

(Dr. William Boyd, Winnipeg.) Dr. Mudd asked whether there was a sap stream to play with.

(Dr. Kunkel.) Yes, there is a sap stream, but it is not comparable to the blood stream; it is not circulating.

THE DIFFERENTIATION OF PLANT VIRUSES BY THE SERUM-PRECIPTIN REACTION. Helen Purdy Beale (by invitation), New York City.

Abstract. If the expressed juice of a plant affected with a filterable virus disease is used to hyperimmunize rabbits, the resultant antiserum contains precipitins, neutralizing and alexin-fixing antibodies.* The serum reactions are specific for the homologous virus extract employed as antigen, and the precipitin reaction may be used to great advantage in the recognition of new host plants, in the detection of "carriers" and in the differentiation and classification of viruses. Plant viruses which are regarded as distinct on the basis of symptomatology, host range, properties, methods of transmission and plant immunity tests, are likewise distinguishable by the serum reactions. Qualitative and quantitative investigations of the serum-precipitin reaction indicate a close association between active virus and antigen.

Discussion

(Dr. Arthur William Grace, New York City.) I should like to ask Dr. Beale in what animal the serum was produced. That was not quite clear to me from what she had to say.

(Miss Suskind, New York City.) I should like to know how purification of the virus was carried out. Was it by filtration only or were chemical methods also employed?

(Dr. Beale, closing.) In answer to the first question, the rabbit was used. I also neglected to say there are neutralizing bodies in the antiserum.

In answer to Miss Suskind, the methods of purification are chemical and also by the use of the Seitz filter.

TITRATION OF ANTIBODIES IN SERUMS OF PERSONS RECEIVING ANTIRABIC TREATMENT. Leslie T. Webster and (by invitation) J. R. Dawson, Jr., New York City.

Abstract. A mouse protection test has been developed for titrating antirabic substances in serums. Street rabies virus passed intracerebrally in Swiss mice 3 to 15 times is diluted, mixed with the undiluted test serum and injected intracerebrally into Swiss mice. Mice receiving mixtures of normal sera plus final dilutions of virus as high as 10^{-6} succumb to rabies after consistently uniform incubation periods. Repeated tests with the same and different normal sera and with the same and four different strains of virus have given uniform results except that early passage virus results in relatively long incubation periods in injected mice.

The protection test is being used to measure the development of antirabic substances in the blood of humans following antirabic treatment. Serum from an individual 2 and 15 years after treatment with "T," "T," and "L" vaccines protected completely against 100 lethal doses of 4 strains of mouse passage virus. This serum diluted 1:50 showed complete protection against 10 lethal doses. Of serums from three individuals tested on the 1st, 8th, 14th, 21st and 30th days

* Beale, Helen Purdy. The serum reactions as an aid in the study of filterable viruses of plants. *Contrib. Boyce Thompson Inst.*, 1934, 6, 407-435.

of treatment with "N" Semple vaccine, one of the 30 day serums showed slight protection, while the remainder were practically negative. Serums from three individuals 8 months after a 21 dose treatment with the same "N" vaccine and from one individual 8 months after a 28 dose treatment with "N" vaccine showed no protection. On the other hand, three pools of 5, 5, and 4 serums from fourteen persons on the 14th day of treatment with "G" Semple vaccine protected against 10 lethal doses, and serums from five of these same persons tested after 37 days, and five tested after 62 days protected fully against 100 lethal doses of virus. And finally, serum from an individual given "H" vaccine 13 and 5 years ago protected against 100 lethal doses.

The "N" and "G" vaccines injected into Swiss mice failed to incite rabies; the "H" vaccine so treated did bring the animals down with rabies.

Discussion

(Dr. Ralph D. Lillie, Washington.) May I ask what type of antirabic vaccines was used in these cases, — whether phenol-killed or the old Pasteur vaccine?

(Dr. Webster, closing.) All vaccines thus far tested have been of the phenol-killed Semple type, with one exception. In this case the "H" vaccine was dried and diluted.

ON THE PROBLEM OF IMMUNIZATION AGAINST POLIOMYELITIS. E. W. Schultz
and (by invitation) L. P. Gebhardt, Stanford University, Calif.

Abstract. Passive immunization of monkeys with large doses of a high titer immune serum provides some protection against subsequent intracranial or intranasal inoculations with virus. For protection against a given dose of virus a disproportionately high antibody titer is necessary. A relatively low protection is afforded against virus administered by the intranasal route. This may be explained by the fact that the olfactory nerve, the usual portal of entrance, is very accessible to the virus and cannot be effectively guarded by immune substances in the blood plasma.

Results obtained following artificial active immunization with variously prepared vaccines indicate that it is much easier to stimulate the formation of antibodies than it is to alter the susceptibility of neurons. Since a true acquired active immunity to poliomyelitis is basically a cellular rather than a humoral phenomenon, the criterion of successful active immunization must be an unquestionable increase in tissue resistance, rather than the appearance of antibodies. A well defined increase in neural resistance seems difficult to obtain even when living virus suspensions are used as vaccines.

PATHOLOGICAL AND IMMUNOLOGICAL PROBLEMS IN THE VIRUS FIELD.* Thomas
M. Rivers, New York City.

Abstract. The most interesting pathological problems in the virus field have to do with the relation of the viruses to host cells. It appears that these active agents are obligate parasites and induce certain changes in the parasitized cells. Inclusion bodies often result from such an infection and a study of some of these structures has yielded valuable information. For instance, it has been clearly shown that certain inclusion bodies represent aggregates of minute structures which either are the virus or are closely associated with the virus. Hyperplasia,

* Presented at special request of the Council.

hyperplasia followed by necrosis, and necrosis are the most important phenomena in the pathological pictures produced by viruses. Inflammation is of secondary significance. In view of this fact one can easily understand why viruses enter into any discussion concerning the etiology of tumors.

Viruses are antigenic, and as antigens and because of the fact that an infection with them is usually followed by a lasting immunity they naturally have attracted the attention of workers in the field of immunology. One would like to know why it is so difficult to obtain a solid immunity with completely inactivated viruses. Also the differences in the duration of immunity produced by active and inactive viruses give rise to certain questions. Active viruses usually lead to a more or less prolonged resistance in a recovered host, while the immunity—often incomplete—induced by an inactive virus may be fleeting or at least of a relatively short duration. Moreover, in a few instances infections with viruses do not produce a lasting immunity.

Is it likely that there are special principles of immunity applicable only to virus diseases? There is no evidence that such is the case. The questions raised above might apply equally well to immunological phenomena in general, whether excited by bacteria, spirochetes, protozoa, or proteins of different sorts. The amounts of antigen, the multiplicity of antigens in an infectious agent the labilities of which may vary, the intimacy of contact of the host with the infectious agent, and the duration of this contact all play a part in the immunological phenomena produced by all infectious agents. Certainly some of the immunological phenomena observed in virus diseases may, therefore, be explained upon the intimate type of parasitism exhibited by the viruses, the lability of certain antigenic components of these agents, and finally, the prolonged sojourn of the viruses in a host once infected.

EXPERIMENTS ON THE EPIDEMIOLOGY OF PSEUDORABIES. Richard E. Shope (by invitation), Princeton, N. J.

Abstract. Pseudorabies is a highly fatal but non-contagious disease in cattle and the common laboratory animals. It is a relatively mild but highly contagious disease in swine. It has been shown that in swine the nose serves both as the portal for the entrance and the exit of the virus. Furthermore, it has been found that fatal pseudorabies infections in rabbits can be induced by merely bringing their abraded skin into contact with the noses of infected swine. The blood serums of swine on two farms where pseudorabies had occurred among the cattle were studied and found to be capable of neutralizing pseudorabies virus. It is believed that in these instances the swine had suffered a mild and unrecognized pseudorabies infection and had probably transmitted their disease to the cattle with which they were associated.

To obtain information as to the incidence of pseudorabies among Middle Western swine, 23 samples of anti-hog cholera serum, representing blood serum from over 2500 swine, were studied. Twenty-one of these samples were found to contain pseudorabies virucidal antibodies. In 9 of the samples the antibody titer was such as to indicate that at least 5 per cent of the animals furnishing the serums had suffered an earlier infection with pseudorabies virus. The virucidal titer of the remaining 12 samples was such as to suggest upwards to a 50 per cent previous pseudorabies infection among the swine supplying the serums. The serums of 23 out of 25 adult swine of Middle Western origin contained pseudorabies virucidal antibodies. Serums obtained from local swine have been found

to be free of virus-neutralizing antibodies. It is concluded from the serological data that pseudorabies may be a highly prevalent, even though unrecognized, disease among Middle Western hogs. The experiments presented suggest that swine may serve as the source of infection for cattle, virus transmitting from the noses of infected hogs to the abraded skin of cattle.

VARIATIONS IN NEUROINVASIVENESS OF CERTAIN VIRUSES IN RELATION TO THE AGE OF SUSCEPTIBLE HOSTS. Peter K. Olitsky and (by invitation) Albert B. Sabin and Herald R. Cox, New York City.

Abstract. This study indicates that as an animal matures it may acquire resistance to certain neurotropic viruses, which depends neither upon previous exposure to infection and subsequent development of humoral antibodies, nor upon any change which age induces in the body as a whole, but rather upon a modification in certain special parts of the nervous system. The observations were made with the Indiana and New Jersey strains of vesicular stomatitis virus which are highly neurotropic in mice. The results in brief are as follows: (1) while young mice, 15 to 20 days old, readily develop a fatal encephalitis when the virus is dropped into the nose, old mice (about 1 year old), with only rare exceptions, show no signs of disease when given as much as 1000 to 10,000 times the minimal amount of virus which is fatal for the young ones; (2) yet young and old mice are equally susceptible when the virus is injected directly into the brain; (3) the resistance of the old mice is not dependent upon the presence of antiviral bodies in the blood; (4) the resistance becomes appreciable between the 20th and 30th days of life; (5) mature mice reveal a similar resistance when the virus is given subcutaneously instead of intranasally; (6) mature mice exhibiting no signs of disease following intranasal administration of virus, nevertheless develop specific humoral antibodies and a specific active resistance to intracerebral inoculation of the virus.

The development of the specific immunity in the mice which showed no signs of disease suggested that an unrecognized infection had occurred somewhere in the body. No virus was found in the blood, lungs, liver and spleen, thus excluding these as foci of infection. Since a direct inoculation in the brain is equally fatal to young and old, it appeared that the difference observed between the different age groups when the virus is introduced by way of the nose might be due to a resistance encountered by the virus somewhere along its course between the nasal mucosa and the important centers in the brain. It remained to determine whether the barrier was pre-ganglionic, *i.e.* in the olfactory nerve fibers with a resultant inability of the virus to reach the olfactory bulb or ganglion; or post-ganglionic, when the virus might reach the olfactory bulb but be unable to spread to the rest of the brain. Experiments revealed that apparently the resistance is chiefly post-ganglionic in the sense just indicated. Whereas in young mice the virus from the nose extended first to the olfactory bulb and then rapidly to the rest of the brain, in mature mice it also invaded the olfactory lobe but failed to spread to the rest of the brain.

It should be pointed out that with viruses which have a high initial invasiveness for the host, like Eastern equine encephalomyelitis in mice, and pseudorabies in guinea pigs, no appreciable difference was seen in their behavior in animals of different ages. It is not improbable that the well known resistance of older age groups in the case of poliomyelitis in man may at least in part be due to the phenomenon just described.

Discussion

(Dr. William Boyd, Winnipeg.) Dr. Rivers, in the remarkable review he gave this morning, pointed out that not very much attention had been paid to the histological or cytological changes in our morning symposium. I should like to ask Dr. Sabin if any histological investigations were made. Does one find in the non-immune, that is to say, in young animals, definite changes, either inflammatory or in the nature of inclusion bodies, and are these absent in the adult?

(Dr. Stuart Mudd, Philadelphia.) One of the slides seemed to imply that you could get passive protection in young mice with the serum of adult mice. Is that true?

(Dr. Sabin, closing.) Histological examinations were made, but unfortunately this is a virus disease which does not give rise to very characteristic cytological changes. No inflammatory changes, other than some dubious increase in cells in the olfactory portion of the brain, were found. The brain and nasal mucosa of the adult resistant mice were sectioned, but they showed nothing which would aid in determining the focus of virus action.

In reply to Dr. Mudd, the slides showed that there is a passive protection in that adult mice which had exhibited no signs of the disease developed antibodies in their serum which protected young mice against 1000 infective doses by the cerebral route.

(Dr. Mudd.) Does the serum of normal adult mice confer protection on young mice?

(Dr. Sabin.) Normal adult mouse serums had no antibodies for the virus.

STUDIES ON INCLUSION BODIES OF A NEUROTROPIC VIRUS IN VARIOUS ORGANS.

Abner Wolf and (by invitation) Margaret Holden, New York City.

Abstract. The W virus was originally isolated by Drs. Gay and Holden from a case of fatal ascending myelitis in a laboratory worker bitten by a monkey. A series of rabbits were injected with this virus in the parotid and submaxillary glands, adrenals, liver, spleen, kidney, testis and skin. In each of these organs lesions were produced which were characterized by necrosis, inflammation with occasional hemorrhage, and in all but the liver and spleen intranuclear inclusions. Hyperplasia was noted in the skin and connective tissue. In each case the infection spread to the nervous system. It involved the spinal cord first when the abdominal viscera or abdominal skin was injected, as evidenced by the limb paralysis; or the brain directly when the parotid or submaxillary were injected, as shown by convulsions. In those instances in which the abdominal organs were injected there was a peritonitis, intense in most cases and slight in only one. Blood vessels in the necrotic organs, in particular the adrenals, showed an intense arteritis with intranuclear inclusions. Connective tissue, subcutaneous fat and striated muscle about the salivary glands were affected. There were intense inflammation and necrosis in the former two and intranuclear inclusions and moderate degeneration were observed in the latter. The nerves in the organs injected always showed inflammatory changes in the perineurium and often endoneurium; occasionally they exhibited necrosis, and at times intranuclear inclusions were found in the Schwann and endoneurial cells.

Intranuclear inclusions were most frequent in the adrenals and salivary glands; fairly common in the central nervous system and rare in the kidney, testicle and skin. They were also encountered in striated muscle, blood vessel

walls and the Schwann and endoneurial cells of nerves. They were not found in the liver and spleen in our animals although seen in 1 case in the connective tissue cells of the markedly infiltrated capsule of the latter.

They varied considerably in type and intensity of staining reaction. In the adrenal they were homogeneous, deeply eosinophilic, oval or spherical, and tended to fill the entire nucleus. The chromatin was margined in every case. Occasionally a partial halo could be seen about the inclusion bodies. In the salivary glands they varied from multiple small inclusions to single large bodies which always had a halo about them. They were rather lightly eosinophilic and somewhat irregular, rather than oval or spherical. In general they were coarsely granular rather than homogeneous. In the kidney and seminal vesicle the inclusions resembled those seen in the salivary glands. In the skin some of the epidermal cells contained small, irregular, deeply eosinophilic intranuclear inclusions which were much like those seen in glial cells to be described later. They were either large, single and homogeneous, or multiple and separated often by bars of chromatin. As found in striated muscle, the inclusions were single, small, very deeply eosinophilic, homogeneous, oval or spherical, and had halos about them. In Schwann and endoneurial cells they resembled closely the inclusions seen in the adrenals, while in blood vessels they were more like those seen in muscle.

In the nerve and glial cells, the spinal cord and brain, inclusions of all the varying types described above were seen. In general the inclusion bodies in the nuclei of the nerve cells were homogeneous, deeply eosinophilic and had halos about them. Very frequently they filled the entire nucleus, as in the adrenal. Occasionally they were granular and irregular, the granules being rather uniform in size. Often they were small, compact, deeply staining and multiple. The cytoplasm of the affected nerve cells was always very pale staining and lacked density. In one instance this change was found in the cytoplasm of nerve cells in the basal ganglia without intranuclear inclusions. The animal had had cerebral symptoms, but showed neither infiltration nor inclusion bodies.

In the glia cells the inclusions were homogeneous and filled the nucleus or had a slight halo. Frequently, however, they were small, irregular, multiple and separated by bars of chromatin, as in the skin. In one rabbit injected in a number of abdominal viscera, there was the usual inception of nervous symptoms by a limb paralysis. There were no inflammatory changes in the spinal pia arachnoid or spinal cord. Numerous intranuclear inclusions associated with degeneration were observed in the glial and nerve cells of the cord, however.

The so-called B virus, isolated from the same human material by Dr. A. B. Sabin, is probably identical with the one here described.

Discussion

(Dr. Albert B. Sabin, New York City.) This virus, the pathology of which Dr. Wolf has just described, is a very interesting one. It was isolated simultaneously from the same case by Drs. Gay and Holden, and by myself. They called it W virus; I called it B virus. I see by this work that the two viruses which we have studied individually are apparently identical, but at one time they expressed the opinion that their W virus was herpes. I should like to know whether further studies since then have caused them to change their minds, and whether the description just given is for herpes or for a distinct virus. I have since then published the results of an extensive biological and immunological investigation

which showed that while the B virus is related to herpes and pseudorabies, it can be distinguished clearly from them by serological and biological methods. One of the important biological distinctions from herpes is that the B virus is pathogenic for the *Macacus rhesus* monkey. It may be recalled that the human disease followed a bite by a rhesus monkey. It has been possible to show that apparently the B virus causes a mild natural disease in the rhesus monkeys, while when transmitted to more susceptible hosts it produces a disease and lesions of the same type seen in man and the rabbit. It belongs to a group of viruses which might be termed pantropic, because of their pluricellular affinities.

(Dr. William Boyd, Winnipeg.) Are the lesions in all the organs purely degenerative, or did those in the brain show any inflammatory changes in addition to the degeneration?

(Dr. Wolf, closing.) In answer to Dr. Sabin, to my knowledge there has been no further work by Gay and Holden on the question of the relation of this virus to the herpes virus. My own interest in the problem is in the pathology and from that point of view I consider that this W virus is identical with the B virus.

In the majority of the organs inflammatory changes were present. But these were not marked and did not form an important part of the picture. In one rabbit intranuclear inclusions were found throughout the cells of the spinal cord, but no inflammatory reaction at all.

SHWARTZMAN PHENOMENON IN VACCINE VIRUS LESIONS. Lewis Henry Koplik
(by invitation), New York City.

Abstract. Recently Gratia and Linz* elicited the Shwartzman phenomenon by combined intradermal injections of testicular vaccine virus and intravenous injection of *B. coli* culture filtrate. It was considered of interest to determine whether vaccine virus cultured *in vitro* would have a skin-preparatory potency and, furthermore, whether the intravenous injection of vaccine virus preparations following an intradermal injection of the culture virus would elicit a local response at the site of vaccination.

Vaccine virus was cultured according to the method of Rivers (Rivers, T. M., *J. Exper. Med.*, 1931, 54, 453-461, and Li, C. P., and Rivers, T. M., *J. Exper. Med.*, 1930, 52, 465-470). *B. typhosus* culture filtrate was made as described by Shwartzman (Shwartzman, G., *Proc. Soc. Exper. Biol. & Med.*, 1929, 26, 843-845). Stock rabbits were used. They were each injected with 0.25 cc. of cultures of vaccine virus fresh or glycerinated. From 1 to 8 days later they received an intravenous injection of *B. typhosus* culture filtrate, of vaccine virus culture or of neurovirus. The effect of repeated intradermal injections of vaccine virus cultures was also noted.

Following the intravenous administration of *B. typhosus* culture filtrate (20-40 units per kilo), of culture virus (1-2 cc. per kilo), or of neurovirus (0.1 cc. per kilo) to rabbits vaccinated with culture virus 3 to 6 days previously, there was a marked change in the appearance of the local lesions in such rabbits as compared with control rabbits. The vaccinia vesicles and pustules became surrounded in 5 to 20 hours by areas of hemorrhage into the skin and subcutaneous tissue. These changes were observed in 60 per cent of the animals so treated but not in the controls. This phenomenon was not observed when the intrave-

* Gratia, A., and Linz, R. *Compt. rend. Soc. de Biol.*, 1932, 108, 238.

nous injection followed the intradermal after 1 or 2 days or in the healing state (*i.e.* after 7 or 8 days). A second intradermal injection of culture virus after an interval of 3 days at the site of the first did not produce visible hemorrhage around the vaccinia lesion. Culture virus heated to 58° C. for 10 minutes was also ineffective in preparing the skin or in producing a state of local reactivity when given intravenously. Glycerine alone did not act as a skin-preparatory factor, and testicular extract caused only a localization of the vaccine virus, if intradermal inoculation of such extract was followed within 6 hours by an intravenous injection of the virus.

From our results it is evident that vaccine virus cultures are effective in preparing the skin of rabbits for the Shwartzman phenomenon when the intradermal inoculation of such cultures is followed after an interval of 3 to 6 days by an intravenous injection of similar culture virus, of neurovirus or of *B. typhosus* culture filtrate. The virus must be potent and an active lesion is essential for the elicitation of hemorrhage at the local site.

PATHOLOGICAL ASPECTS OF THE LOCAL AND GENERAL SHWARTZMAN PHENOMENON. I. E. Gerber (by invitation), New York City.

Abstract. Detailed histological studies of the local Shwartzman phenomenon were previously made by Karsner and Moritz, Apitz, and Kielanowski and Selzer. The present study was undertaken in order to determine the sequence of events in the appearance of the hemorrhagic reaction in the skin. Experiments were performed with bacterial filtrates of ascertained skin-preparatory potency in various dilutions. The skin preparation was followed by intravenous injections after various intervals of time. The actions were studied at various periods. It was observed that no parallelism existed between the degree of inflammation following skin preparation alone and that following the appearance of the phenomenon. A slight degree of preparatory inflammatory reaction may be followed by a marked hemorrhagic and inflammatory response upon intravenous injection of the bacterial filtrate. The reaction in the skin after the intravenous injection is out of proportion to the inflammation that results from mere augmentation of the preparatory inflammatory reaction by repeated skin injection alone.

Apitz recently described the general Shwartzman phenomenon obtained by means of two successive intravenous injections of bacterial filtrate 24 hours apart. Each intravenous dose varied from 0.5 to 5.25 cc., or were given so that a total of 0.7 cc. was administered in 3 divided doses on the 1st day and a total of 6.3 cc. in 3 divided doses on the 2nd day. The internal organs showed diffuse vascular and concomitant degenerative changes. In the present study a series of rabbits received two successive intravenous injections of bacterial filtrate, with an interval of 24 hours between the injections. The potency of these filtrates was ascertained by their ability to elicit the local Shwartzman phenomenon. The doses ranged from 50 to 500 reacting units, *i.e.* 0.005 to 1 cc. Some rabbits also received skin-preparatory injections simultaneously with the first intravenous injection. Widespread pathological alterations were observed in the internal organs. These consisted of diffuse vascular changes in the kidneys, lungs, and liver, and apparently concurrent degenerative changes in the heart, liver and kidneys.

Discussion

(Dr. Eugene L. Opie, New York City.) It seems to me doubtful if these experiments exclude the possibility that inflammation has a significant part in the production of the Shwartzman phenomenon. The tests that are described show that substances causing the Shwartzman phenomenon are inflammatory irritants. The experiments of Menkin and a number of others have shown that some readily recognizable substance, such as trypan blue injected into the circulation, localizes in an inflamed area when the dye is used to produce a conspicuous blue spot. If a similar process occurs with the reactions that have been described, the vaccine of the second injection may accumulate at the site of the first injection and reaching it by way of the vascular system may produce the hemorrhage and thrombosis that accompany the Shwartzman phenomenon.

(Dr. Howard T. Karsner, Cleveland.) I am very much gratified that Dr. Opie made the statement that he did, and I hope that what he had to say will allow Dr. Gerber to continue what he undoubtedly thought of in connection with the general Shwartzman phenomenon. However, I do not think that what Dr. Gerber said alters the statement which has been made by several people, including Moritz and myself, that the inflammation which succeeds upon the injection of the protective factor is different from that which follows the primary injection, only quantitatively and not qualitatively.

(Dr. Gregory Shwartzman, New York City.) I should like to draw Dr. Opie's attention to the fact that a number of various substances of non-bacterial origin were tested by me and other investigators. Not a single one was shown thus far to produce the necessary state of reactivity in the rabbit. Dr. Freund reported in a recent publication that silver nitrate, which produces primary hemorrhages, also elicits the state of reactivity in the guinea pig. The author did not state whether he tested this preparation in rabbits. So far, I have tested 45 rabbits with various dilutions of silver nitrate. Silver nitrate usually produces a primary necrotic lesion, but no effect is produced by the subsequent intravenous injection of a potent bacterial filtrate upon the prepared site.

The various non-bacterial substances which were used for skin preparation are those capable of eliciting the severest type of primary inflammation (turpentine, arsenic, and so on); those producing acute vasodilatation, vasoconstriction, and vasoparalysis (histamine, adrenalin, acetylcholine, calcium chloride, urethane ethyl, and so on); antigen-antibody complexes (horse serum plus anti-horse rabbit serum, eggwhite plus eggwhite antiserum, and so on), various substances blocking the reticulo-endothelium system and the like. None of approximately 60 of the various non-bacterial substances employed ever produced any state of reactivity. In recent work, Kielanowski excised small portions of skin at various periods of time after preparatory injection. Rabbits were then given the provocative injection. The remaining non-excised portions of the prepared site served as a control as to the susceptibility of the animal to the phenomenon. In his extensive histological studies he found that the degree of primary inflammation bore no relationship to the reaction produced by the subsequent intravenous injection. Moreover, Apitz studied the inflammation produced by bacterial filtrates in animals refractory to the phenomenon (rats). It is extremely interesting that the degree of primary inflammation observed was the same as in susceptible animals. In addition, there are a number of various bacterial filtrates which produce a considerable degree of primary inflammation

(staphylococcus, streptococcus, pneumococcus filtrates, and so on) and yet completely fail to elicit the state of reactivity.

It is perfectly true that fixation of particulate matter by inflamed tissues clearly postulated by Dr. Opie may play an important rôle in the localization of the toxin from the blood stream. Naturally, the reacting factors must localize in the tissue in order to produce an effect, but there is no doubt whatsoever that there must be recognized also a peculiar type of a reactivity in the tissues elicited by special bacterial factors. This reactivity is quite apart from the incidental inflammation. Furthermore, there must be recognized also some additional dynamic factors which would be responsible for the production of the dramatic lesion following their localization.

(Dr. Theodore J. Curphey, New York City.) I should like to ask Dr. Gerber whether he made any platelet counts on the rabbits at the height of the reaction in an attempt to explain the thrombi that they show.

(Dr. Gerber, closing.) In answer to Dr. Curphey, we have not done any hematological studies on these animals.

TRANSMISSION EXPERIMENTS OF THE VIRUS OF POLIOMYELITIS IN MICE. Maurice Brodie, Samuel Goldberg and (by invitation) Phyllis Stanley, New York City.

Abstract. The mouse was used in this work owing to the fact that the virus of poliomyelitis survives for a longer time in the mouse brain than in that of the other small laboratory animal.

Three series of mice were subjected to 7 or more short doses of X-ray, following which they were injected intracerebrally and intraperitoneally with poliomyelitis virus. In the first series on the 11th and 12th day after injection the mice were slow, weak and had ruffled hair. Inoculation into mice and a monkey of a suspension of the brains of several of these mice produced no reaction. The second series of mice showed symptoms similar to those of the preceding series after the same interval of time following injection of the virus. Untreated mice injected with a suspension of these brains showed identical symptoms but with a shorter incubation period. The virus has been carried through 17 generations in mice, during which time the incubation period has become shortened to 3-4 days, the infectivity of the virus for the mouse increased and the symptoms have become more clear-cut. The injection of the suspension of brain material from the original mice of this series, into a monkey, produced a rise in temperature, but in the next passage in mice gave what seemed like typical poliomyelitis. Passage 3 also gave the typical disease in monkeys, while with passage 7 and 8 specific neutralization was obtained with three specimens of serums having poliomyelitis antibodies.

The mice of the third series behaved like those of the second. Passage of their brain material into untreated mice produced similar symptoms. A suspension of their brains produced a typical poliomyelitis when injected into a monkey and on the 2nd passage up to a 1:5000 dilution and on the 8th passage to a 1:1000 dilution of the mouse brain. During the transmission of the virus of this series through 14 generations of mice, it has undergone changes in incubation period, infectivity and symptoms produced, identical with those of the preceding series. Specific neutralization was obtained up to the 16th passage.

In the mouse, the disease produced by the injection of this virus is acute. It is evidenced by irritability, ruffled hair, ataxia, humped back, convulsions, twisting

of head and death. In the mouse, the pathology is found mainly in the brain and meninges. In the subarachnoid space it consists of mononuclear infiltration which is mainly perivascular. Perivascular collars, areas of hemorrhage, focal areas of necrosis and glia reactions are seen in the cerebrum. The spinal cord and brain stem show an occasional perivascular collar and some hemorrhagic foci. The cerebellum appears normal. The distribution of the virus shows some correlation with the histopathological picture. We appear to have transmitted the virus of poliomyelitis for the following reasons:

1. The virus has been successfully transmitted from mouse to monkey and from monkey to monkey and mouse.
2. Serum from convalescent and actively immunized monkeys and humans neutralized the virus.
3. The neutralizing power of various serums appeared to correlate when tested both in mice and in monkeys.

In the 17th passage and thereafter of both series the mice came down in the usual incubation period and with what appeared to be the same symptomatology. However, the disease incitant was no longer neutralized by poliomyelitis neutralizing substance, nor did it produce paralysis in monkeys.

Discussion

(Dr. J. Furth, New York City.) Is the application of repeated small doses of X-rays preferable to a single massive dose? It has been found in our laboratories that a single massive dose of hard X-rays increases susceptibility to several agents and inflicts profound damage upon the blood-forming organs. To understand the mechanism of increased susceptibility to the virus of poliomyelitis, it would be significant to know whether soft X-rays are necessary to produce it.

(Dr. Albert B. Sabin, New York City.) I should like to ask two questions: first, is the mouse passage virus pathogenic for guinea pigs and rabbits, and second, is a monkey convalescent from monkey passage virus resistant to mouse passage virus, and is a monkey convalescent from mouse passage virus resistant to monkey passage virus?

(Dr. Edwin W. Schultz, Leland Stanford University, Calif.) I should like to ask whether the anterior horn cells showed neuronophagia.

(Dr. Brodie, closing.) We do not know whether a single large dose of X-ray would be any better than repeated doses, because we did not try it. The only thing we tried to do was to get a block of the reticulo-endothelial system by a single large dose of India ink.

We have not been able to infect guinea pigs or rabbits, which I did not mention, with the virus.

In reply to Dr. Sabin's question, a monkey that recovered from mouse passage virus showed neutralizing substance for the monkey passage virus; we did not inoculate this animal directly intracerebrally, inasmuch as it died soon after being bled. Monkeys immunized with mouse passage virus showed specific antibodies which neutralized the monkey passage virus.

In reply to Dr. Schultz, even though the mice occasionally showed what appeared to be paralysis, they never showed sufficient in the spinal cord to account for it. We found no neuronophagia in the spinal cord. The only lesions we have found are mononuclear infiltration of the subarachnoid space and some perivascular infiltration in the gray and white matter, and some hemorrhage, but no nerve cell destruction. The spontaneous mice virus which we picked up looked

more like polio than the other because we found what appeared like definite destruction in the spinal cord.

TRANSMISSION OF EQUINE ENCEPHALOMYELITIS BY MOSQUITOES. Carl Ten Broeck and (by invitation) Malcolm H. Merrill, Princeton, N. J.

Abstract. Since in the East the great majority of cases of equine encephalomyelitis are found along the shore line, the ability of a number of salt marsh mosquitoes to transmit the disease has been tested. *Aedes sollicitans*, the salt marsh mosquito found most abundantly in the regions where the disease occurs, can be infected by feeding on infected brain mixed with blood, or on an infected guinea pig or horse. It apparently retains the virus as long as it lives and regularly transmits it by biting. Transmission of the virus from infected to normal guinea pigs and from infected horses to normal guinea pigs and a horse has been obtained.

Further experiments, not so extensive, indicate that *Aedes cantator*, *Aedes taeniorhynchus* and *Aedes vexans* will also act as transmitting agents. The last is a fresh water mosquito which may be involved in the transmission of the Western type of the disease. Transmission experiments using *Culex pipiens* and *Anopheles quadrimaculatus* have been uniformly negative.

In making transmission experiments it is important that mosquitoes be fed on material containing virus of high titer. When the titer is low the virus can be demonstrated immediately after feeding by inoculation of a suspension of crushed mosquitoes, but inoculation and feeding experiments at 5 days and thereafter are negative.

Discussion

(Dr. Marshall Hertig, Boston.) I should like to inquire the method by which the mosquitoes were fed the suspension of brain and blood.

(Dr. Ten Broeck.) The mosquitoes were starved for 4 days and kept for 24 hours without water, after which the virus was offered them and they took it readily.

(Dr. Hertig.) How was it applied?

(Dr. Ten Broeck.) On a piece of cotton.

FURTHER STUDIES ON THE INFECTIVITY OF TRACHOMA. L. A. Julianelle and (by invitation) R. W. Harrison, St. Louis, Mo.

Abstract. Studies conducted in this laboratory reveal that trachoma is an infectious disease transmissible to monkeys. The specificity, course and nature of the experimental infection, together with the natural resistance of certain animals to trachoma, their lack of acquired immunity to the disease following infection, the inability to establish the infectious agent permanently in monkeys, and so on, have already been reported. Since that time the investigation has been devoted almost exclusively to the determination or demonstration of the causative agent of trachoma. The general method employed has been that of elimination and up to the present the following is the information gained from this study.

It was established very early that faulty or deficient diet plays no accessory part in the causation or evolution of the disease. Elaborate studies of the bacteria cultivable from trachomatous tissues indicate that the infectious agent is not bacterial, whether considered as a single specific organism or as several or-

ganisms associated in a non-specific infection. Further experiments revealed that under the usual conditions of filtration the infectious agent does not traverse Berkefeld filters.

It appeared, then, that the incitant of trachoma resides in that field flanked on one side by bacteria and on the other by filterable viruses. This includes, therefore, (1) the basophilic, heterogeneous, epithelial cell inclusion described by Prowazek and Halberstader, (2) Rickettsiae, (3) non-filterable viruses, or (4) some unknown and unsuspected agent. Epithelial cell inclusions are difficult of reconciliation because materials not containing inclusions are frequently infectious, and materials containing inclusions are frequently not infectious. Furthermore, despite numerous examinations, inclusions have never been found in preparations from monkeys infected experimentally.

Repeated examinations in the human and monkey have revealed no Rickettsiae, and cultivation by tissue culture methods has yielded thus far cultures completely devoid of bacteria or Rickettsiae, and even infectivity.

In attempting to acquire information on the non-filterable virus concept of the disease, it has been found that active trachomatous tissues preserve their infectivity for 2 weeks or more following inoculation in the rabbit and guinea pig testicle. The ground testicle is infectious for monkeys, inducing the typical signs of the experimental disease. The testicular material is bacteria-free; it shows no distinct pathological changes, grossly or microscopically; it exhibits no organisms or inclusions, and on tissue culture yields no growth of infecting organisms as determined by direct examination or inoculation of monkeys. Similar manipulation of normal testicle, or testicle inoculated with material from folliculosis of humans, from chronic conjunctivitis of unknown origin, and even from non-infectious trachoma induce no changes in the conjunctiva of monkeys. Simultaneous inoculation of active or inactive trachomatous tissues and normal testicle does not affect the original degree of infectivity of the material. It is not possible to interpret the significance of these experiments at the present time. The work is being reported not to define the infectious agent of trachoma, but merely to indicate the adoption of a new method in the study of the etiology of this disease. Whether this method will prove fruitful or not must wait upon further work.

THE PATHOGENICITY OF *BRUCELLA ABORTUS* FOR WHITE MICE. William H. Feldman and (by invitation) Carl Olson, Jr., Rochester, Minn.

Abstract. For the purpose of determining the pathogenicity of the cattle and swine varieties of *Brucella abortus* for white mice, 3 different strains of *Br. abortus bovis* and 3 of *Br. abortus suis* were used to inject a total of 36 animals. The mice were divided into three groups for the purpose of autopsy and one group was killed after 30 days, another after 44 days, and the third group after 70 days. Practically all of the animals survived the respective periods of the experiment and *Br. abortus* was recovered from the spleen of 28 or approximately 83 per cent of the 34 animals in which recovery of the organism was attempted. *Brucella* agglutinins of significant titer occurred in nearly all the animals whose blood was tested. The pathological anatomy was also studied and while gross manifestations of the disease were infrequently seen in the respective mice, characteristic microscopic lesions were present in most of the animals. The lesions which were essentially diffuse or focal accumulations of histiocytic cells of peri-

vascular inception occurred most often in the kidneys and liver. The spleen, testes and epididymis were affected less frequently.

Conclusions

1. Cattle and swine strains of *Br. abortus* when injected intraperitoneally are pathogenic for white mice.
2. Brucella agglutinins are present in the blood of the inoculated animals and the specific organism is recoverable from the spleen.
3. Although grossly visible evidence of a diseased state infrequently occurs, rather characteristic lesions of the kidneys, liver, and less frequently of the spleen, testes and epididymis, may be observed microscopically.
4. White mice should be satisfactory animals for the isolation of *Br. abortus* from spontaneously infected material.

THE RELATION OF ALLERGY, RESISTANCE AND ANTIBODIES IN ANIMALS VACCINATED WITH THE CALMETTE-GUERIN BACILLUS (B. C. G.). B. J. Clawson, Minneapolis, Minn.

Abstract. Allergy and resistance were studied in relation to each other and in relation to antibodies in the serums of animals vaccinated with B. C. G.

Allergy as discussed refers to the phenomenon illustrated by the skin tuberculin reaction.

Resistance was indicated by a partial or complete retardation of the progress of infection due to virulent strains of tubercle bacilli.

The antibodies studied were agglutinins, complement fixation antibodies, opsonins and lysins.

Methods and Materials: (A) *Allergy.* Rabbits were vaccinated so as to produce allergy in some but not in others. Allergic rabbits were desensitized and the antibody content of the desensitized rabbits studied. A method of injecting animals to produce a degree of allergy and a minimum or no measurable antibody content in the serums was also employed.

(B) *Resistance.* Resistance due to vaccination was determined by comparing the degrees of tuberculosis in normal and vaccinated animals following an inoculation with a lethal dose of a virulent strain of the tubercle bacillus. The antibody content was studied in vaccinated resistant animals.

(C) *Relation of Allergy to Resistance.* The three methods used for this study were: (1) to vaccinate animals so as not to produce allergy; (2) to wait for the allergy to disappear before giving the virulent injection; and (3) to compare the duration of allergy and resistance.

Results of Experiments: There appeared to be no definite proportionate or necessary relation between the presence of allergy, as manifested by the intravenous tuberculin skin reaction and antibodies in the blood.

Definite resistance against virulent injections was developed by vaccinating rabbits and guinea pigs with B. C. G. The antibody titers were uniformly increased in the vaccinated resistant animals.

No proportionate or necessary relation appeared to exist between the phenomenon of allergy and the immune state (resistance).

The degree of the titers of the antibodies tended to correlate the degree of resistance.

Discussion

(Dr. Max B. Lurie, Philadelphia.) I am very much interested in hearing Dr. Clawson's statement of the lysin effects on tubercle bacilli *in vitro*. It has not been stated whether the effects are due to the cells or to the serum in the system. Certainly if you take highly immune serum, if you take the sera or plasma of extensively tuberculous rabbits highly immune to tuberculosis, and incubate that with tubercle bacilli, you find that they actively support the growth of the bacilli even to an extent greater than that of normal plasma. Is it due to the cells or to the plasma in the mixture?

(Dr. Joseph D. Aronson, Philadelphia.) I am in accord with Dr. Clawson in that there may be no relation between allergy and resistance. However, I cannot agree that there exists a relation between resistance and antibodies. A number of years ago I carried out a series of experiments on goats and sheep, which were bronchoscoped and small amounts of tubercle bacilli were then sprayed into the trachea at irregular intervals. For a period of several years serological reactions and the tuberculin reaction were carried out on these animals. No correlation could be established between the antibody content, the sensitivity to tuberculin, the longevity of the animals and the extent of tuberculosis. The animal that lived the longest and had marked fibrosis of the tuberculous lesions of the lung had the smallest amount of humoral antibodies. I have not succeeded in modifying the course of tuberculosis in guinea pigs treated with large amounts of immune serum obtained from goats or sheep hyperimmunized with the tubercle bacillus. Nor was the course of tuberculosis modified by the injection of such antisera previous to injection of guinea pigs with virulent tubercle bacilli.

(Dr. L. Dienes, Boston.) I made some observations which correspond to those of Dr. Aronson. By the treatment of tuberculous guinea pigs with virulent tubercle bacilli strong antituberculous serums were produced which gave complement fixation with tubercle bacilli in the dilution of 1:2000 or higher. As large doses as 15 to 20 cc. of these serums exerted no protective action in guinea pigs. I have seen also that very strong allergy produced with killed tubercle bacilli is not necessarily associated with increased resistance. The slower or faster progress of the disease and the resistance to new infection seem to depend on many specific and non-specific factors. Probably some of these factors are yet unknown; their way of cooperation is also unknown. At present it seems best to keep our judgment in suspense in the problem of tuberculosis immunity.

(Dr. E. T. Bell, Minneapolis.) Whatever theoretical considerations there may be against Dr. Clawson's work, we must not lose sight of this main fact—that the great majority of these animals were completely protected against massive inoculations of tubercle bacilli, the guinea pig against the human strain, the rabbit against the bovine strain. The controls were riddled with tuberculosis, but the vaccinated animals for the most part showed no lesions whatever.

(Dr. E. L. Opie, New York City.) It seems to me that our methods of measuring sensitization are unsatisfactory because we depend on a skin reaction in which a variety of factors is involved. We use tuberculin prepared by prolonged heating, and very different from the antigenic substances associated with the living tubercle bacillus. In the reactions used to measure antibodies several factors may be involved, and though two of these factors may remain constant, another factor may vary. Hence we should not expect an exact complete parallel in the occurrence of the reactions that are associated with sensitization and

resistance in tuberculosis. I should like to know what was the method of measuring lysis.

(Dr. E. M. Medlar, Mt. McGregor, New York.) I should like to ask Dr. Clawson how long he kept his animals. Tuberculosis is a disease of long standing, and you cannot draw definite conclusions from experiments in tuberculosis unless you keep your animals for some time. Recently I have had rather an interesting result in which I inoculated guinea pigs with living B. C. G., and kept them isolated where there was no chance of their becoming contaminated with any other tubercle bacillus. I forgot all about them. They had been isolated for $2\frac{1}{2}$ years when I finally sacrificed them. One of the animals was riddled with tuberculosis. It appeared perfectly healthy at the time of killing.

(Dr. Stuart Mudd, Philadelphia.) Had these B. C. G. cultures been maintained according to Calmette or had they been altered?

(Dr. Medlar.) It was an original Calmette culture which we received in 1929. This culture has always been grown on a bile-glycerin-potato medium, as recommended by Calmette.

(Dr. Clawson, closing.) In reply to Dr. Lurie's question, I first will answer Dr. Opie as to how lysis was determined. It probably may not be correct to say that I had a humoral antibody. The lysis here took place within the phagocytic cells. The cells were obtained by injecting 100 cc. of paraffin oil into the peritoneal cavity of a normal rabbit. In 4 days the material was taken out, which gave a very heavy suspension of almost pure mononuclear leukocytes. Equal amounts of those cells, plus serum at a dilution of 1:75, plus a measured amount of B. C. G. were put on a mixing wheel and rotated for 1 hour in an oven at 37° C. Then a measured amount was put onto a measured surface on a slide and fixed with methyl alcohol and stained with the acid-fast stain. The determination of the lysis was made by determining the absence of the organisms by acid-fast or non-acid-fast stain. I realize this is a rather crude method, and do not depend on it very much unless there is a decided change. A 50 per cent reduction was considered certainly significant. The lysis does not take place in the serum. The serum without the cells did not reduce the number of B. C. G.

Dr. Aronson brought up a few points which I think I should make some statements about. The fact that antibodies are not detectable in serum does not prove the absence of the potentialities of antibodies. I find animals which will not show antibodies at the time of protection. If they are injected intravenously and then examined the next day for antibodies, the antibodies will come back up almost as high as they were normally. This I think cannot be due to a new development of antibodies.

In regard to Dr. Opie's statement as to the methods of measuring, I realize the methods of measurement in all the work on allergy and the concentration of antibodies are very crude. However, with the animals I used 1 mg. of Old Tuberculin; it is of such potency that when 0.05 of a mg. is injected intracutaneously into a positive individual a strong reaction is elicited.

In answer to Dr. Medlar's question, how long do I keep the animals, I presume he refers to the time I keep the animals after injection with B. C. G. The animals are kept from 3 weeks to as long as 5 months after being injected with B. C. G. and never did I find any progressive lesions. I did find microscopic lesions in the lungs in the animals which were injected intravenously with living B. C. G. The other animals were kept from 90 to 110 days, and those that survived were killed.

BACTERIAL LOCALIZATION AND GROWTH IN NORMAL AND IMMUNE TISSUES.

Paul R. Cannon, Chicago, Ill.

Abstract. The object of these experiments was to determine the comparative ability of certain organs of normal and of actively immunized animals to remove living bacteria from the blood stream and to ascertain the effects of immunization upon the growth proclivities in the different organs. The method was to excise the organs aseptically at varying periods of time after the intravenous injection of the bacteria, followed by incubation at 37° C. for 22 to 24 hours, in order to allow bacteria present in a tissue at the time of death of the animal to grow into colonies. The tissues were then fixed, sectioned and stained to demonstrate the bacterial colonies. Broth cultures of *Staphylococcus aureus*, *B. typhosus* and pneumococcus type I were used in order to avoid the introduction of masses of microorganisms into the blood stream, injected in equal amounts, simultaneously, into a normal and an immunized rabbit. The animals were sacrificed at intervals of from 20 minutes to 24 hours.

The results may be summarized as follows:

1. Bacterial colonies of *Staphylococcus aureus* and *B. typhosus* developed readily in the liver, spleen, lungs, kidneys and blood of both normal and immune animals. The colonies were most numerous in the liver and spleen, and in the earlier stages much more numerous in the immune animals, showing both the concentrating activity of these organs and the ability of the bacteria to withstand any bactericidal action of tissue fluids to the extent of preventing abundant growth. In fact, staphylococci were able to grow in the blood within the cardiac ventricle of immune animals injected intravenously at least 18 hours before the animal was sacrificed. There was very little tendency for colonies to appear in the kidneys or lungs, although they were present in animals killed comparatively soon after intravenous injection, but in negligible numbers, as compared with those observed in the liver and spleen.

The conclusions drawn are that bacteria tend to accumulate and concentrate in the immune liver and spleen in greater numbers than in the normal liver and spleen under comparable conditions. Furthermore, the bacteria can withstand the bactericidal activity of the blood and tissue fluids to a considerable degree, and after the death of the cells can grow into colonies. Colonies do not develop in many organs with a rich blood supply, such as the lungs, kidneys, pancreas or myocardium, thus showing the relative inertness of ordinary capillary endothelium as a phagocytic tissue. The results indicate the predominant influence of phagocytic cells in the removal of bacteria from the blood and suggest the inadequacy of the blood and tissue fluids as bactericidal agencies after the death of the cells themselves.

Discussion

(Dr. Max B. Lurie, Philadelphia.) I was very glad to hear the report of this paper because for several years I have studied the fate of tubercle bacilli in various organs after intravenous inoculation of rabbits. By culturing known quantities of the different organs on egg media, and then determining the number of colonies that developed from a given tissue, it was found that 24 hours after injection the localization was exactly as Dr. Cannon has reported. The quantity distributed in the different organs is as follows: The greatest amount per weight of organ is found in the spleen, next to that in the liver, next to that the bone

marrow, which Dr. Cannon has not studied, next to that in the lung, and least of all in the kidney.

(Dr. E. T. Bell, Minneapolis.) Dr. Cannon no doubt realizes that this localization of the bacteria is due to the distribution of the reticulo-endothelial system. The liver and the spleen contain the greater part of this. The reticulo-endothelial cells take up the bacteria, which are held there. The absence of bacteria in the kidneys is due to the fact that the endothelial lining of the glomerular capillaries is extremely thin; there is practically no cytoplasm in any of these cells in the normal kidney. There is nothing in the glomeruli that can act as a phagocyte. Whenever a bacterium is found in the glomerulus it is either mechanically stuck to the capillary wall or taken up into a polymorphonuclear in the blood. This is the distribution of India ink when it is injected in small quantities.

(Dr. Stuart Mudd, Philadelphia.) I should like to ask whether there is a difference between survival and later history of the bacteria in the normal and immune animals.

(Dr. Theodore J. Curphey, New York City.) Was there any tendency for localization of the pneumococci?

(Dr. Cannon, closing.) In reply to Dr. Curphey's question, I would say that the use of pneumococci in this work was not very successful as the organisms would not grow into colonies but grew diffusely. I saw no tendency, however, for localization in the lung as compared with other organs.

In regard to Dr. Mudd's question, these animals were all sacrificed within 24 hours. My chief object was to see how bacteria would grow in the excised organs of immune animals rather than to observe variations in bacterial localization, but inasmuch as this method demonstrated localizing potentialities of different organs, such as Dr. Lurie has just mentioned from his work, and as we have found also by determining the germ-content of tissues or organs of animals injected intravenously with living bacteria, I thought it was interesting to see the correlation from another angle of approach.

The lack of phagocytic power of glomerular endothelium, as mentioned by Dr. Bell, is of interest and is not generally realized. A paper appeared on this subject within recent years in which the method of staining bacterial colonies after death in excised tissue was used. The author observed that when animals were killed after 10 minutes and others after 4 hours, more colonies were found in the kidneys at the end of 10 minutes than at the end of 4 hours, and concluded that this demonstrated a greater phagocytic power in the kidney in the 10 minute animal than in the 4 hour animal. It seems to me, as Dr. Bell stated, that the explanation is quite obvious: the organisms are circulating and being removed from the blood as they go through the liver, spleen and bone marrow, and being washed out of the kidneys, until eventually practically none is left in the kidney from which a colony can develop. The point I have been interested in is that endothelium, not only in the kidney but elsewhere, as in the pancreas, thyroid, muscle, and so on, is relatively inert, so far as its ability to engulf bacterial particles is concerned.

ON THE MECHANISM OF IMMUNITY IN TUBERCULOSIS. THE FATE OF LIVING TUBERCLE BACILLI WITHIN A LOCALIZED AGAR FOCUS AND THEIR DISSEMINATION IN THE BODY OF NORMAL AND IMMUNIZED RABBITS. Max B. Lurie, Philadelphia, Pa.

Abstract. Sterile 6 per cent agar in saline at pH 7.4 is melted, and when cooled to 48° C. is thoroughly mixed with a suspension of virulent tubercle bacilli in India ink or trypan blue. A portion is injected subcutaneously into normal and B. C. G. vaccinated or actively tuberculous rabbits; the other portion is cultured on egg media. At varying intervals after inoculation, the number of living tubercle bacilli present in a given amount of the agar coagulum and its surrounding capsule, as well as that present in the draining lymph nodes and internal organs of the normal and immunized rabbits, is determined. The fate of the bacilli is then correlated with the histological changes in the corresponding tissues.

It was found that the agar coagulum is broken up into particles by an exudate of fluid, fibrin and cells, and that the bacilli grow freely in the agar totally away from the cells in the normal animal. In the immunized animal they either do not grow at all or are markedly inhibited in their multiplication in this location. Under the same conditions tubercle bacilli impregnated in agar and kept at body temperature die completely in 11 days. These facts point to an extracellular inhibitory factor in immunity to tuberculosis. Yet agar mixed with plasma of normal or tuberculous rabbits equally supports the growth of the contained bacilli *in vitro*.

In the normal animal the bacilli grow dispersed as well as in large, loose colonies. In the immunized animal they either persist in their original form without multiplication or grow as minute, dense clumps. This clumping is at least partly non-specific, for the same relations obtain with particulate matter such as carbon, which is largely agglutinated in dense masses in the tuberculous animal, whereas in the normal animal the carbon particles are largely dispersed and the agglutinated masses are less frequent and of looser texture. This is to be correlated with the greater retention of carbon at the site of reinfection than at the site of primary infection.

The bacilli in the normal animal penetrate the surrounding tissue and multiply unhindered within the cells. In the immunized animal there is little penetration of the capsule by the bacilli and those that do are actively destroyed by the cells.

However, all these factors which tend to prevent the spread of the bacilli from the site of reinfection are insufficient at first, and in the first days, due to the greater intensity of the inflammation and the increased lymph flow, more tubercle bacilli reach the immediate regional nodes in the reinfected than in the normal animal. But, due to the greater capacity of the cells to destroy the micro-organism, its growth is markedly retarded and they soon disappear in the immunized animal while they multiply unhindered in the normal animal.

The deeper lymph nodes and the internal organs of the sufficiently immunized animal practically completely destroy the few invading bacilli that reach them, whereas the large numbers invading those organs in the normal animal continue to multiply.

Discussion

(Dr. Paul R. Cannon, Chicago.) I should like to ask Dr. Lurie what is his impression of the cause of the greater extension of the tubercle bacilli to the regional lymph nodes in the tuberculous animals as compared to the normal.

(Dr. Calvin G. Page, Boston.) What kind of egg culture medium was used?

(Dr. Lurie, closing.) The explanation for the more rapid dissemination of the tubercle bacilli from the agar focus in the tuberculous animals is to be associated with the much greater degree of inflammation at the focus and the much greater flow of lymph from the site of reinfection in the tuberculous animal, as compared with that in the normal; this increased lymph flow tends to sweep away the bacilli with it. The fixation which is apparent later does not operate effectively in the early stages of the tuberculous reinfection.

In reply to Dr. Page's question, I have used Lowenstein's medium as a base, in which bone marrow infusion replaced the distilled water, as required by the original formula of Lowenstein.

CULTURAL AND PATHOGENIC PROPERTIES OF A NEW PATHOGEN ISOLATED FROM HUMAN CASES OF MENINGO-ENCEPHALITIS. Caspar G. Burn (by invitation), New Haven, Conn.

Abstract. An unidentified bacillus has been isolated during the past 18 months at the New Haven Hospital from four individuals, three infants and one adult. It was isolated both clinically and at postmortem in pure culture in the three infants. In the case of the adult it was found to be in association with a pneumococcus type III. The pathology consisted of multiple foci of necrosis and exudation in the liver and occasionally a similar lesion was found in the other organs. Two of the individuals showed central nervous system involvement consisting of hemorrhage and suppurative meningo-encephalitis.

The organism is a Gram-positive, non-spore-forming bacillus appearing singly, in clumps and occasionally in short chains. The colonies on blood agar plates resemble those of a hemolytic streptococcus, differing, however, in that the colonies are larger, flatter and more translucent. The usual sugars are fermented without gas, except for a delayed fermentation in lactose and glycerin.

The results with animal inoculations vary with the species of animal employed, the number of bacilli introduced and with the route of inoculation. Intravenous injection in rabbits varying from 100,000 to 10 million per cc. resulted in paralysis, convulsions and meningeal irritation on the 4th to 5th day and finally caused death. At autopsy, the liver showed diffuse spottings with focal zones of necrosis. In the central nervous system, the lesions revealed an extensive meningo-encephalitis in which subarachnoid hemorrhage occurred in 20 per cent of these rabbits. Monkeys developed similar liver and brain lesions, but larger quantities of bacilli were required. On the other hand, guinea pigs failed to develop central nervous system involvement upon intravenous inoculation, but instead consistently showed myocardial abscesses which resulted in death in 14 to 20 days.

Carriers were not found in two of the families in which epidemiological studies were made.

Further studies concurring the unusual affinity which these organisms have for the central nervous system are now in progress.

Discussion

(Dr. Edwin W. Schultz, Leland Stanford University, Calif.) I should like to add a few words to what Dr. Burn has said. About a year ago we reported in the *Proceedings of the Society of Experimental Biology and Medicine* similar observations on a case of meningo-encephalitis in a nurse at the Veterans' Hospital at Palo Alto. An interesting feature of this case was that the organism was recovered from the spinal fluid in pure culture 12 times over a period of more than 3 months. The cultural and experimental observations which we made seem exactly like those described by Dr. Burn. The nurse still has residual symptoms. We are carrying out further studies on this organism experimentally. I should like to ask Dr. Burn whether he has demonstrated motility in these organisms. We have not been able to demonstrate any motility.

(Dr. William Boyd, Winnipeg.) Are these sporadic cases?

(Dr. Burn.) Yes.

(Dr. Arthur W. Wright, Albany.) I should like to ask Dr. Burn if serological and immunological studies were made with the organism which he isolated; and also to ask Dr. Schultz if any studies have been carried out with the blood of the nurse who survived the infection, to determine whether or not her blood serum contained antibodies which were specific for the infecting agent.

(Dr. Schultz.) We checked all the cultures which were isolated from the nurse during this period culturally and serologically against sera produced in rabbits. I do not recall the extent to which the nurse's serum agglutinated, but it did in a low dilution eventually.

(Dr. Burn, closing.) In reply to Dr. Schultz' question about the motility, I have also been unable to demonstrate any evidence of motility; with various flagella stains used I have been unable to show any evidence of their presence.

With regard to the serological and immunological studies, we did some, and are carrying out more at the present time. In the rabbits surviving after a 5 day period, that is, those inoculated in small quantities, there will be evidence of agglutinins in the blood of these animals ranging anywhere from 1:20 to 1:100. Also monkeys, though they were more resistant, and larger quantities of bacilli had to be used to produce infection, showed agglutination in dilutions from 1:100 to 1:150 in their sera. We are also carrying out some serological tests with other strains of organisms we believe culturally related to this organism, and believe it agrees in every respect culturally and I think in some respects to the pathogenicity of the organisms described by Murray and Webb which they isolated from rabbits in 1925. Culturally we cannot differentiate them at all. Our strain also produced a definite monocytic response in rabbits within a period of 4 to 5 days, just at the height of the meningitis. Other studies are being made along these lines.

A NEW SPECIES OF THE GENUS *MONOSPORIUM* ASSOCIATED WITH CHRONIC OTOMYCOSIS. David L. Belding and (by invitation) Carl B. Umanzio, Boston, Mass.

Abstract. The association of a species of the genus *Monosporium* with a chronic infection of the ear is recorded for the first time. In the literature this genus has been reported chiefly in connection with Madura foot. The external auditory canal was lined with crusts, fine white scales, and moist macerated tissue. There

was a thin yellow to thick creamy discharge with an offensive odor. The morphology and cultural characteristics of the fungus indicate that it is a species distinct from those that have been reported in man.

THE BACTERIAL FLORA ASSOCIATED WITH FOREIGN BODIES IN THE TRACHEA AND BRONCHI. Carl Joseph Bucher, Philadelphia, Pa.

Abstract. Two hundred and forty-three specimens of mucopus obtained bronchoscopically from the tracheobronchial tree of patients who had a foreign body there were cultured. A study was made of the bacteria recovered in cultures and the type of foreign body, the age of the patient, and the sojourn in the lung, to determine how much importance was to be attached to them. It was concluded that the bacterial flora were less important in this respect than the nature of the foreign body, the degree of obstruction produced, location in the air passages, the sojourn there, and the age of the patient.

Discussion

(Dr. Calvin G. Page, Boston.) I have cultured bronchial mucus from more than 150 cases with the intention of growing fungi, if they were present, using three special sugar media. I should like to ask the speaker if he attempted to cultivate fungi. The cases I studied were mostly of routine bronchoscopy for tumor and so on, and not for the removal of foreign bodies. The idea was to try to find fungi, and I found them in only a few cases.

(Dr. Bucher, closing.) In addition to the organisms reported on the chart, occasionally there were *Monilia*. I suppose they may be classed as fungi. They were the only fungi found. I have cultured several thousand bronchoscopic specimens, and they occasionally occurred.

THE VISCERAL PATHOLOGY IN SCARLET FEVER. Henry Brody (by invitation) and Lawrence W. Smith, New York City.

Abstract. The paper presents a study of 61 autopsy cases of scarlatina and related streptococcic infections. It includes a histological report on the non-suppurative, toxic manifestations of the disease in the various viscera. The lesion is an interstitial one, consisting of an exudate of round cells, including chiefly lymphocytes but also many plasma cells, and other large monocytes. The lesion has been found in almost all of the body tissues. It is not, we believe, the result of direct injury to the interstitial tissue, but rather, primarily, a widespread injury to the vascular endothelium with, secondarily, fluid and cellular exudation.

Lesions of varying severity are found in over 90 per cent of the hearts, in these cases, principally as infiltration about the small coronary arteries or as a subendothelial infiltration of the coronary veins and the endocardium. Similarly, over 80 per cent of the kidneys show these lesions which are primarily of interstitial mononuclear infiltration in the boundary zone between the cortex and medulla. In the more extensive cases the picture resembles, both in gross and microscopically, lymphatic leukemia.

The liver is the next most frequently involved organ, the changes being noted in over 70 per cent of the cases. They are found principally around the smaller vessels in the portal areas, but extend out into the parenchyma to some degree.

Similar lesions about the veins and capillaries have been seen in the spleen, adrenals, pancreas, lung, pituitary, testis, tissues of the pharynx, regional and distant lymph nodes, salivary glands and aorta.

Discussion

(Dr. Virgil H. Moon, Philadelphia.) I have been much interested in the lesions in various organs resulting from scarlet fever infection, and am interested in Dr. Smith's interpretation of them as due to toxic effects rather than as due to the direct infection of the tissues by the organisms. The lesions in the liver which he states are particularly specific call to mind an instance in which in three children in the same family cirrhosis of the portal type developed following scarlet fever. In one of these cases there were such lesions as are shown here, but more marked, leading to typical portal cirrhosis with ascites. In this case streptococci were cultivated from the liver and were demonstrated in large numbers in the sections of the liver. I offer this suggestion, that perhaps in certain cases the lesions described may not be due to toxins elaborated by the organisms, but may be due to the presence of organisms in the tissues. I should like to ask Dr. Smith whether he made examinations to determine if bacteria were present in or about the lesions.

(Dr. Stuart Mudd, Philadelphia.) I should like to ask what the incidence of positive blood cultures was in this group.

(Dr. Otto Saphir, Chicago.) There are two questions I should like to ask. The first is, are the lesions which Dr. Smith showed so beautifully the direct result of whatever causes scarlet fever, or are they the result of the complications of scarlet fever? This might be answered by stating the time interval between the onset of the disease and death. The second question is, did Dr. Smith find any circumscribed proliferative lesions in the myocardium which resemble the so-called Aschoff bodies?

(Dr. E. T. Bell, Minneapolis.) I should like to ask Dr. Smith why he attributes this to endothelial injury. The lesions here are proliferative and exudative, especially in the walls of the arteries. They resemble the lesions which may be obtained experimentally by the injection of streptococci. I see no reason to consider this an endothelial reaction, such as we get in some of the virus diseases, like typhus and Rocky Mountain spotted fever.

(Dr. Howard T. Karsner, Cleveland.) I think that studies such as Dr. Smith has made are of unquestionable value in enlightening us as to the pathology of scarlatina. It occurs to me that the principal lesions he has shown are in relation to the blood vessels, and the study of the vascular system in various types of acute infectious diseases, which has gone on for many years, has shown that in many of these diseases lesions of this general character occur. In reference to arteriosclerosis, one wonders what bearing this sort of inflammation may have on subsequent disease of the vascular system. These lesions which Dr. Smith has shown are of great interest, but when Dr. Smith states that he sees something specific in their morphological character I fail to follow him, and I should like to have him explain further what he means by that. I see Dr. MacMahon sitting here, and I hesitate to speak of the resemblance found in the kidney sections to the picture seen in malignant nephrosclerosis.

(Dr. H. E. Robertson, Rochester, Minn.) I am interested in the after-effects of this disease, because the after-effects may be proof after all of the primary character of the disease process. The other day I examined a boy about 20 years

of age who had had scarlet fever 3 months before, and the outstanding lesion in his case was a thickening of the walls of the blood vessels, almost to the point of occlusion, in a great many organs, particularly the kidneys. This boy had developed hypertension, but had not developed glomerulonephritis. I am wondering if Dr. Smith has had any experience in the later stages of scarlet fever.

(Dr. Norbert Enzer, Milwaukee.) Several years ago we attempted to repeat and confirm Duval's experimental production of glomerular nephritis, and in the course of these experiments lesions were produced in rabbits similar to those exhibited by Dr. Smith. This was particularly true of the kidney lesions. We were unable to stain bacteria in the tissues, but did find bacteria in the blood stream. In these animals we frequently found hyaline thrombi and even cellular thrombi in the afferent glomerular vessels. Also in these animals we occasionally encountered medial necrosis of the medium sized arteries of the kidneys. These lesions were very similar to the early findings in periarteritis nodosa. I should like to know from Dr. Smith whether he found thromboses in the arterioles of the kidney, or evidence of medial necrosis in the vessels.

(Dr. Smith, closing.) Obviously one cannot say much very conclusively in 10 minutes about the details of the work which has been done on this. In reply to Dr. Moon, we have attempted in all of these cases to take cultures of the individual viscera and of the blood stream. In a small percentage of these we have had positive blood cultures. These slides have all been stained for bacteria by methylene blue or Gram's stain, and in no instance where these lesions have occurred have we been able to demonstrate organisms.

In reply to Dr. Mudd's question, the blood cultures ran about 20 per cent positive in these cases, but the visceral cultures have almost always been negative.

In regard to the question of this representing a true scarlatina picture or the results of late secondary complications, I think I can answer that best by saying that I tried to emphasize that in these cases the individuals nearly all died within the first week or 10 days of the disease. As a matter of fact, a number of the slides that I showed came from patients on the 3rd to 4th day of the disease clinically, so that we are dealing with a very acute process which we feel is fundamentally the basis of the scarlet fever infection rather than a secondary late manifestation.

There are occasional instances in the slides of the heart in which we have found fairly circumscribed lesions in the interstitial tissue, but in no instance have we found anything that we were willing to classify as a definite Aschoff body. I have tried to avoid any particular reference to the cardiac pathology because we are expecting to present a comparable study of the rheumatic and scarlatinal hearts at a later date with Dr. Louis Gross.

The endothelial injury we think is pretty clear-cut in these cases, as we have seen it in a good many hundred sections from the very onset of the disease. We found a definite degeneration of the endothelial cells lining the vessels. This is not necessarily either on the venous or arterial side. Both were involved. Perhaps there is a little greater emphasis on the venous side than on the arterial. Then following such damage to these cells we found the usual proliferative change of their vascular endothelium.

I am perfectly willing that Dr. Karsner should take exception to my rather broad statement of specificity because I think that I may have been stretching a point for the sake of emphasis. As far as we are concerned, in making examinations in cases of scarlet fever, diphtheria, measles, pertussis and varicella, and

also in the group of some of the virus infections, particularly poliomyelitis, we have found certain vascular changes which are perhaps in some respects comparable, but we never have seen in any of these other diseases any such definite relationship, with such clear-cut progressive stages in the development of these lesions. The changes are so characteristic that we have been able to make a fairly accurate tentative diagnosis of scarlatina on the basis of histology alone.

In regard to the after-effects, unfortunately all our cases are acute. We have very little opportunity for follow-up work, and we have had no opportunity, therefore, to know what happens to these individuals later in life. It seems to me very probable that there might be some relation between these acute changes and changes that occur later in the blood vessels.

It is quite true that some of these changes might easily be mistaken for acute periarteritis in the initial stage, and in perhaps a half dozen of our cases we have been able to demonstrate acute necrotizing lesions in the vessel walls. In such cases we have found positive blood and tissue cultures, so that we feel there may be some secondary relationship under these circumstances. Where the lesion has been what we might speak of as "Simon pure," we have not been able to demonstrate these acute necrotizing changes, nor to get positive visceral cultures. We have not seen the characteristic thrombi in the afferent capillaries in these cases, nor in our experimental animals.

I do not know just how important these blood vessel lesions may be in respect to late effects, in the kidney particularly, nor the portal infiltration described, to the development of a subsequent cirrhosis of the liver, but it seems quite reasonable that such extensive changes as we have demonstrated may well be significant factors in the pathology of the heart, the liver and the kidney in later life.

THE PATHOLOGY OF CHRONIC ULCERATIVE COLITIS. H. E. Robertson, Rochester, Minn.

Abstract. Chronic ulcerative colitis owes many of its peculiarities to its long duration with alternating periods of quiescence and repair and periods of relapse. Deep ulceration, even to perforation, extensive inflammation and scarring of the submucosa, hemorrhage, hypertrophy of the muscular coats, and irregular new growth of the epithelial layer constitute the usual pathological picture. Repair of the mucosa on a pathological submucosa often gives disorderly polypoid growths which tend to become carcinomatous. The frequency with which carcinoma complicates this disease, even in young adults, is an outstanding example of the effect of chronic inflammatory processes on the development of cancer.

Discussion

(Dr. Paul R. Cannon, Chicago.) I should like to ask Dr. Robertson what is his interpretation of these linear ulcerations, how frequently he observed them, and what he thinks of their pathogenesis.

(Dr. E. T. Bell, Minneapolis.) What is the incidence of bacillary dysentery in Dr. Robertson's experience, as compared with this non-specific colitis?

(Dr. Max B. Lurie, Philadelphia.) What do the blood vessels going into the polypi show? Do they show fibrosis?

(Dr. Robertson, closing.) Replying to the last question first, the blood vessels going into these polypi show no particular changes.

There has been, so far as I am aware, no instance of the bacillary type of dysentery. In about half the cases the organism described by Bargaen has been isolated in pure culture.

The longitudinal ulcerations I think come about by the contraction of the colon in which a part of the mucosa is protected.

A PRELIMINARY REPORT ON *INTRA VITAM* BIOPSY STUDIES OF THE PATHOGENESIS OF PNEUMOCOCCUS LOBAR PNEUMONIA. T. J. Curphey, Brooklyn, New York.

Abstract. Biopsy sections of the lungs of patients suffering from lobar pneumonia were obtained from a series of recovered cases at different stages of their disease as well as from cases at postmortem in which autopsy was not permitted. The method used was the punch biopsy of Hoffman. The object of the study was to determine whether the histogenesis of this process differed in recovered cases from that usually seen in postmortem studies. From the material thus far available, certain definite changes can be noted in the pericapillary histiocytes in the early stages of the disease, which would suggest that these cells play a definite defensive rôle. Certain interesting changes are similarly noted in the alveolar capillaries. These preliminary observations tend to stress the need for further *intra vitam* studies, in more cytological detail, of the mesothelial elements of the lung in lobar pneumonia.

Discussion

(Dr. Arthur W. Wright, Albany.) I have two questions I should like to ask Dr. Curphey. To me the introduction of a trocar into an infected focus in a lung seems a rather dangerous procedure. I should like to ask how often after the biopsy specimen has been taken from a pneumonic lung secondary infections such as acute pleuritis, which perhaps later developed into empyema, have occurred, and whether or not there is serious danger of such secondary infections. In the second place, after seeing the photomicrographs which were thrown on the screen, I should like to ask if the method as used so far has shown anything that has not yet been demonstrated in pneumonic lungs obtained at autopsy. Many of us would like to know more than we do about the pathogenesis of lobar pneumonia and this method may offer a means of studying the early changes that occur in this disease, but I fail to see that as yet anything new has been learned. In my opinion this technique is not only too dangerous for the patient, but at present seems to offer too little in the way of added knowledge of the pathology of the disease to justify its use.

(Dr. Eugene L. Opie, New York City.) I have been interested in secondary infections that occur in association with pneumonia, particularly when it follows influenza, and I have been impressed by the readiness with which *Streptococcus hemolyticus* invades a preëxisting lobar pneumonia. In the presence of secondary infection with *Streptococcus hemolyticus*, abscess formation and empyema might follow the introduction of a needle large enough to remove lung tissue.

(Dr. Curphey, closing.) In answer to Dr. Wright's question, I may say that we do not think the incidence of empyema is any greater in the group biopsied than in a series of non-biopsied cases, based of course on physical findings and X-ray evidence. The point we are trying to make about the use of this method is that we realize the risk, of course. I stressed that. We feel very definitely that

the pathology of recovery of lobar pneumonia cannot be studied on the autopsy table. That was the prime motive for instituting this method.

I think Dr. Opie's point is well taken. I unfortunately am not able to give him any definite answer; I cannot say how frequently streptococcus infections follow these cases.

PULMONARY CHANGES DUE TO THE ASPIRATION OF LIPIDS AND MINERAL OIL. Irving Graef, New York City.

Abstract. Since Laughlen's report (1925) and Pinkerton's clinical and experimental observations (1927 and 1928) of pulmonary inflammation associated with the deposit of oils and fats, an increasing number of instances of this condition have appeared in the literature.

We have studied 6 cases — 3 in infants (aged 6 months, 16 months, and 18 months respectively) and 3 in adults (aged 54, 66, and 70), in which oily material was demonstrable in considerable quantity in the lungs. In 4 instances an unsaponifiable oil (liquid at room temperature) was identified in the pulmonary deposits, and in 2 there was a mixture of fats and fatty acids.

Pulmonary suppuration was present in 4 instances. The presence of oily substances was anticipated in the lung on macroscopic examination twice. The lesions in the cases associated with mineral oil are strikingly similar in that the substance was dispersed in fine droplets, usually intracellular in location, and occupied the interstitial tissue at the expense of the alveolar spaces. In many instances lobular architecture was obliterated with a few rudimentary sacs as the only indication of respiratory parenchyma. The oily material in these cases was also found in intra-alveolar macrophages; multinucleated foreign body giant cells containing fat droplets were found in 1 case. In the same case there were macroscopic lesions which were mistaken for tumor metastasis at close examination (this patient had a recurrent adamantinoma of the mouth). The nodules were composed of dense fibrous tissue containing free globules of oil and a rich intercellular deposit which resembled adult adipose tissue. There was a marked accumulation of the offending substance around the blood vessels and bronchi. In the infants there was a diffuse increase in reticular and collagenous fibers around the distended oil-containing cells.

In 1 instance in which the aspiration of large quantities of neutral fat and fatty acids was inferred, there was striking necrosis of the lung resembling caseous necrosis in tuberculosis. An acid-fast membrane similar to the familiar hyaline membrane of other pulmonary lesions was demonstrated around air bubbles in the aspirated material. Another case associated with the deposit of neutral fat and fatty acids was found on reviewing a case of bronchogenic carcinoma with a broncho-esophageal fistula and multiple bronchiectatic abscesses with marked fibrosis in the appended lobe. In the walls of the cavities and in the proliferative interstitial tissue there were abundant deposits of fatty substances identified as true fats and fatty acids.

Lipid analyses were done on material from 4 of the 6 cases. The amount of total lipid, total unsaponifiable and saponifiable material, and the cholesterol content were determined in samples yielding large amounts of unsaponifiable material. Several control specimens showing pneumonia, chronic passive congestion or no lesions were also analyzed. Identification of the unsaponifiable substance supported the histochemical examination in 2 of the 4 cases associated with mineral oil (there was not sufficient material in the third for analysis

and the fourth had been preserved in alcohol). In the case with massive aspiration of fat and free fatty acids, showing necrotizing pneumonitis with the peculiar formation of an acid-fast membrane, the saponification number and the iodine number were done and indicated that milk fat and the fatty acids of butter fat were probably present. A qualitative test for cod liver oil yielded positive results in this case as well.

Histochemical examination is an even more satisfactory method of identifying the offending substance, because it can be localized as well. Failure to reduce osmic acid or to react with Nile blue sulphate, the absence of anisotropic globules and a yellow stain with scharlach R serve to identify mineral oil. The reduction of osmic acid, the appropriate reaction with Nile blue sulphate, and orange red or salmon red reactions with scharlach R indicate the presence of a neutral fat or fatty acid. Formation of an acid-fast membrane indicates the presence of "blown" fatty acids described by Pinkerton in rabbits given oils rich in free fatty acid. The absence of anisotropic droplets rules out the presence of cholesterol or cholesterin esters. Solubility in lipid solvents may also be used.

Clinical data confirmed the use of the offending substance in considerable quantities in 5 of the 6 cases. Particular attention should be paid to the cases of mineral oil deposit because of the wide use of this substance as a vehicle for medication introduced in the nasopharyngeal passages and as an intestinal lubricant.

Mechanisms by which this substance gains entrance into the trachea and lung are not clear. Being non-irritating on the surface of the pharyngeal mucosa, it does not incite the cough reflex. The presence of mild anesthetic substances, like menthol in some preparations, may also enhance their passage into the trachea. Primary defective action of cilia in chronic infections of the respiratory tract, or the loss of the cough reflex in weak and debilitated individuals may also play a rôle.

Discussion

(Dr. Andrea Saccone, New York City.) This wonderful presentation by Dr. Graef has been corroborated by Roussy and Besançon, in the last number of the French Archives of Pathological Anatomy; in this article the authors are describing the possible pathology of lipiodol injected into the lungs. They emphasize very much that while vegetable oil does not produce any pathological lesions in the lung of the experimental animal, the mineral oils are responsible for extensive pathological conditions in the lungs.

(Dr. D. Murray Angevine, New York City.) I have had an opportunity to autopsy 2 of these cases at the New York Hospital. The first case was diagnosed clinically and at autopsy presented a characteristic picture; never having seen a case of oil pneumonia before, it was easily recognized when suspected. On scraping the lung tissue at the autopsy table oil droplets were readily found. The second case was not diagnosed clinically, but at the autopsy table, being aware from the appearance of the lung that some oily substance might be present, the unmistakable odor of cod liver oil was readily detected, showing that the sense of smell may be of value in making this diagnosis. Even after fixation in formalin one could detect the odor of fish oil. Several observers who had not seen the lung at autopsy examined it later and detected the odor of cod liver oil. Color tests for the presence of vitamin A were done.

I do not think that Dr. Graef has brought out the fact clearly enough that in most of these cases the individuals are in a debilitated condition. In our cases,

one child had been in a plaster cast for 10 months, and another child had a definite hydrocephalus with degeneration of the basal ganglia.

I should like to ask one question. One of your cases showed a large amount of fat apparently only in the mononuclear cells. We did frozen sections stained selectively for fat on several cases of uncomplicated bronchopneumonia, and were surprised at the amount of fat found in the mononuclear phagocytes in these cases. Was Dr. Graef's experience similar?

(Dr. Paul R. Cannon, Chicago.) I have studied 1 case of aspiration of cod liver oil which was of interest from the standpoint of the pathogenesis in that the trouble seemed to start from the forcing of the oil. The parents held the nose of the child and forced the child in spite of much resistance to take the cod liver oil, and the symptoms dated from that period. The lesions were more advanced than those shown by Dr. Graef in that three large cavities appeared in the lungs. These were demonstrated by X-ray before death.

In regard to the mechanism of the process in the lungs, I believe the mechanism may be much simpler than is usually supposed. I have taken normal rabbits and dropped a 50 per cent emulsion of cod liver oil into their nostrils, and have been able to demonstrate the oil in the alveolar spaces within 48 hours. Fischer-Wasels reported finding about 100 cc. of mineral oil in the lungs of an elderly woman dying with extensive pulmonary fibrosis. He found that this woman had been in the habit for 20 years of using mentholated mineral oil as a nasal spray and that she had bought it in large quantities from her druggist. It seems to me this subject is particularly important because of the extensive advertising over the radio and in the newspapers on the use of oil droplets for respiratory infections, and undoubtedly this condition has been much more common recently. We have had 6 cases which we believe were of oil aspiration, but did not study them particularly until we encountered this rather dramatic one of cod liver oil aspiration.

(Dr. Alan R. Moritz, Cleveland.) I should like to ask Dr. Graef if fat stains on the mediastinal lymph nodes give any indication that the oil was being mobilized.

(Dr. E. T. Bell, Minneapolis.) Dr. Graef's photomicrographs bring out a point which a great many of us have no doubt seen, the fact that the alveoli are lined by columnar epithelium. I should like to ask if he has studied the origin of these cells. There is quite a body of opinion among histologists that the lung alveoli have no epithelial lining, yet in these cases of lipoid pneumonia nearly everyone finds this appearance: the alveoli lined by typical epithelial cells, usually columnar.

(Dr. Stuart Mudd, Philadelphia.) In the course of direct observations on *in vitro* phagocytosis Mrs. Mudd and I have seen rather striking differences between the macrophages and the polymorphonuclear leukocytes in that the macrophages readily ingest mineral oil, and the polymorphonuclear cells do not. I take it that Dr. Graef found the mineral oil in macrophages. Did he observe it in the polymorphonuclear leukocytes, and if not, was the same difference between the two types of phagocyte observable with other types of oil?

(Dr. Alfred Plaut, New York City.) I wonder whether the distribution of the oil might partly be merely a physical process. Oil diffuses on dry surfaces. When we put oil in a container, we will find that it climbs up and the next day oil will be found on the outside also. I do not know whether on a wet surface oil can be diffused, but it might be interesting to look for oil in the accessory nasal sinuses.

(Dr. Mudd.) Oil cannot spread over a wet surface. However, it may be the trachea under certain circumstances is not so wet; I do not know.

(Dr. H. Edward MacMahon, Boston.) From Dr. Graef's paper one might be led to believe that whenever a group of alveoli filled with lipoid-containing cells is found, this material must be of exogenous origin, reaching the lung by aspiration. I should like to point out that it is a fairly common observation, both in gross and microscopically, to find large and smaller areas of lung parenchyma adjacent to chronic suppurative lesions in the lungs and especially in the neighborhood of a malignant tumor in which the alveoli and also, though to a less marked degree, the interstitial tissue are rich in large, swollen, desquamated epithelial cells and mononuclear cells filled with lipoid. Such findings are extremely variable, at times occupying wide tracts of lung tissue, and resemble so closely the lesions described in this paper as to be indistinguishable from them. In so far as Dr. Graef and others who have discussed this paper have made no mention of the possibility of an endogenous source of lipoid material, namely, from retention, degeneration and a disintegration of cells within the lung, I should like to emphasize the importance of the endogenous source of lipoids in contrast to the exogenous material obtained by aspiration. I believe the former source to be equally important in the etiology of lipoid pneumonia, and in adults, at least, the more common.

(Dr. Norbert Enzer, Milwaukee.) In only 1 case shown by Dr. Graef were multinucleated giant cells found. This, I believe, was in an adult with esophageal bronchial fistula and a chronic interstitial fibrosing pneumonia. It will be interesting to know whether Dr. Graef had observed giant cell formation in the children; also whether the fat surrounded by the giant cells was the same as that phagocytized by the macrophages.

(Dr. Kornel L. Terplan, Buffalo.) From some of the pictures Dr. Graef showed, it seems as if atelectasis has been one important pathogenetic factor, following the occlusion of bronchioli by the aspirated oil.

In the 1 case that I saw at the Buffalo Children's Hospital, the gross picture of the lungs was very characteristic. There was a distinct yellow color to the pulmonary parenchyma shining through the pleura. Appearance and consistence resembled more that of a chronic atelectasis with induration than a real pneumonia. The color was a peculiar mixture between the dark blue of atelectasis, and chrome yellow; it was entirely different from the color of lipoid-granulation tissue, as seen in chronic pneumonia.

(Dr. Graef, closing.) The first point, in reference to the relative safety of the use of lipiodol in visualization of the bronchial tree, is that there is abundant evidence in the literature indicating that iodized vegetable oils produce practically no lasting pathological changes in the lung.

With reference to Dr. Angevine's remark about debility predisposing to this type of pneumonia, it has been the experience at Bellevue that aspiration pneumonias in general occur in debilitated and weak children. Nevertheless, in our 6 cases, 2 of the adults and 1 child were not debilitated or congenitally defective. Furthermore, in cases reported by other observers, Pinkerton particularly, it is worth knowing that some cases may show the clinical picture of an acute pulmonary infection, and only at autopsy may the presence of an oil or lipid material be noted. An example of this sort may be anticipated when massive aspiration of pharyngeal contents takes place at one time.

The finding of very occasional macrophages containing fat droplets in sections

from ordinary pneumonia is not unusual — especially in infants who may aspirate from their milk diets as a terminal or incidental event.

In reply to Dr. Moritz's question, occasionally macrophages in the lymphatics and the septa could be found containing oily material, but the amount of deposit in the mediastinal lymph nodes was extremely small and discovered with some difficulty.

We have also noted that the oily material was never deposited within polymorphonuclear leukocytes. When intracellular it was always in macrophages.

In reply to Dr. Bell's question, we have not systematically studied the origin of the lining cells of the alveoli in these cases.

As to Dr. Plaut's remarks in regard to the climbing phenomenon exhibited by oily material, I might mention that during our chemical analyses some of the recovered oil was left in a beaker overnight, some of which was found on the outside of the beaker the next morning.

The lipids found in cases in which true fats or fatty acids are demonstrated with careful histochemical methods may be endogenous (the use of scharlach R alone, of course, is not differential). Certainly in the 1 case of bronchiogenic carcinoma in which we found lipids in the walls of abscesses and supporting tissues, we realized that some of this material might have come from necrotic tumor. I make no claim as to its source, but wish to point out that this patient had a broncho-esophageal fistula, was on a fluid diet rich in fats, and the lipid-containing lesions were found only in the lobe appended to the fistulous bronchus.

We examined incidentally the lungs in 6 other cases of bronchogenic carcinoma with the idea that when these patients had been bronchoscoped the operators may have used mineral oil as a lubricant. These cases showed no lipids or oil-containing lesions.

If an alveolus is found stuffed with what appear to be fat-containing cells and the material dissolves scharlach R, but fails to reduce osmic acid, and is unsaponifiable on extraction, a paraffin oil of exogenous origin must be implicated.

The question of the formation of the multinucleated giant cells interested us because we had expected from Pinkerton's report that such cells might be found in the juvenile cases in which cod liver oil or mineral oil were implicated. They were not found in these, and I was left with the idea that the 1 adult case that did exhibit the foreign body giant cells had had the process for a far longer period than the infants. This was true in Pinkerton's experimental mineral oil lesions.

In reply to Dr. Terplan, primary atelectasis was not noted. What he termed bronchioles, I think, were non-muscular tubes which I believe represent cross-sections of alveoli with swollen lining epithelium. This formation is due to the increase in interstitial contents at the expense of the alveolar air space.

STUDIES IN EXPERIMENTAL OLEOTHORAX. D. H. Saley (by invitation), H. S. Willis and (by invitation) Lucia Ellwart, Northville, Mich.

Abstract. Several indications for the use of gomenolized oil have been given by clinicians who have used this product in the treatment of pulmonary and pleural tuberculosis. The results of the clinical use of oleothorax show considerable diversity and there is need of further knowledge of its effect.

This experiment was planned to ascertain the action of certain oils when injected intrapleurally in rabbits. Over 100 animals have been used. Paraffin and

cotton-seed oil alone and with gomenol were used in varying dosage. Efforts were made to determine if oil migrated from the pleural space, and if so, by what mechanism. Tieman's soluble blue was added to the oil as an emulsion and the retromanubrial lymph nodes, parietal pleura, lungs, diaphragm, liver and spleen were examined in gross and microscopically at varying intervals for evidence of the dye. Efforts were made to prevent the formation of adhesions by the oil by injecting amniotic fluid.

Pneumothorax was established in normal rabbits and oil introduced into the space, in some instances in one dose; in others the oil was given in weekly doses for 5 or 6 weeks.

The early response was the same to all oils introduced and consisted of a rather marked outpouring of fibrin which was deposited on all pleural surfaces. Soft, fibrinous adhesions were seen as early as a week after injection, and later these became firm, dense and widespread. In some specimens the heart and lung were glued to the sternum and thoracic wall by dense, massive adhesions. The diaphragm on the injected side showed typical elevation and thickening. Occasionally the retromanubrial lymph nodes became enlarged.

Cotton-seed oil tended to be rather quickly absorbed, while paraffin oil persisted in the pleural space indefinitely. Cotton-seed oil tended to form an emulsion with the fibrinous exudate, and the adhesions which it caused tended to become less dense as the oil was absorbed. Neither the amount of oil injected nor the proportion of gomenol used modified the final pathological finding; one injection caused practically the same effect, in regard to adhesions, as repeated injections.

Smears and cultures from material obtained intrapleurally were repeatedly sterile and one specimen with blue in the oil showed the retromanubrial nodes sacculated with oily pigment.

It was thought for a while that "Amfetin" facilitated absorption of adhesions, but further work made this highly problematical.

Histologically the area of reaction consisted of fibrin which embraced globules of fat and a few polymorphonuclear leukocytes and lymphocytes, giving the impression of granulation tissue. Later the cells became increased in number and finally the areas of reaction assumed a definitely more fibrous appearance. In instances where cotton-seed oil had been absorbed the adhesions and exudate underwent more or less absorption.

Discussion

(Dr. Andrea Saccone, New York City.) The Doctor reported that before the injection of oil into the pleura some air had been injected, and in the slide projected there was some injury to the alveolar wall. I should like to ask if any of the pathology of the alveoli could be produced by the previous insufflation of air in these cases.

(Dr. Howard T. Karsner, Cleveland.) I wish to confirm Dr. Willis' observation by observations I made in the course of studies of pulmonary infarction where active hyperemia of the lung was produced by the injection of mineral oil into the dog's thorax. No air was injected at that time; oil was placed in the lung, and the type of acute inflammatory change corresponds very closely to that shown by Dr. Willis. There was a good deal of phagocytosis of oil droplets, so that macrophages appear in the lymphatics under the pleura.

(Dr. Arthur J. Vorwald, Saranac Lake.) I also wish to confirm Dr. Willis' experiments. Dr. Hayes of Saranac Lake submitted tissue from experimental animals in which he injected the pleural cavity with paraffin oil, gomenol and olive oil to me for examination. In all instances, on examining the animals within comparable periods, he saw no difference in the type of reaction of various oils, and in all cases there was a marked thickening of the pleura, with very little penetration of the lung tissue by the oil.

(Dr. Willis, closing.) In reply to Dr. Saccone's question about the effect of air alone, I should like to say that the lung was collapsed by air being put into the thoracic cage rather than into the lung itself, and that when the lung was put down by air and oil added to the space the air was then withdrawn so that nothing more than a very transient exposure to the air existed. We did not check carefully the effect of the air alone, because the animal's response to pneumothorax is perfectly well known.

OBSERVATIONS ON THE VOLUME-DIAMETER RATIO OF ERYTHROCYTES IN SOME DISEASES. Theodore R. Waugh, Montreal, Canada.

Abstract. Observations are reported on changes in the shape of the human erythrocyte in various conditions, particularly the anemias. This alteration in shape is disclosed by comparison of the average corpuscle volume, average corpuscle diameter and an index of the thickness as determined by the formula $V/R^2 = \pi h$. In posthemorrhagic anemias and hypochromic anemia with achlorhydria the cells become smaller and thinner. In pernicious anemia there is a general tendency to a larger but flatter cell, though some cases show an abundance of small thick forms. In familiar hemolytic jaundice the erythrocytes are exceedingly small and thick (spherocytes). In a rare combination of hemolytic jaundice and pernicious anemia, this spherical character of the cells was preserved during reversion to the early embryonic, megaloblastic type.

Discussion

(Dr. Willard S. Hastings, Philadelphia.) I should like to ask Dr. Waugh whether he measured the cells in dry smears or wet mounts.

(Dr. Waugh.) These smears were made dry and stained in the usual manner. As I stated, I appreciate that the figures which one obtains in dry smears are by no means the same as one obtains in wet preparations. At the same time, we feel that the figures are representative of the change as they would be if one used the wet preparation. I emphasized the importance of picking out carefully certain places in the smears, because if one employs thick smears the results of course will be quite erroneous, but if thin smears are used and proper places picked out, one can measure 250 cells in one smear and 250 in another and get approximately the same figure for the average diameter reading.

STUDIES ON THE CELLULAR PATTERN OF BONE MARROW AT ROUTINE AUTOPSY.

Robert J. Williams, Providence, R. I.

Abstract. This study was undertaken in view of the lack of available details concerning the cellular pattern of bone marrow at routine autopsy, using the section technique. In addition it is purposed to emphasize certain facts concerning the distribution of the red marrow in the long bones.

The material consists of marrow from the lumbar vertebra, sternum, the junc-

tion of the lower and middle third of the humerus, femur and tibia from 100 unselected cases in adults. Obvious diseases of the bone marrow were excluded. Cases with leukocytosis and secondary anemia were not excluded. Selection of these bones is based on Seecof's work which will be referred to later. The marrow from each bone was classified grossly as no hyperplasia, slightly hyperplastic, moderately hyperplastic and hyperplastic. This gross classification corresponded approximately to the marrow being fatty, being one-third red marrow, two-thirds red marrow, and being made up entirely of red marrow. The hyperplasia was confirmed microscopically.

The microscopic technique used was essentially that suggested by Custer with Maximow's hematoxylin - azure II eosin staining method. In the case of the vertebra and sternum, however, marrow pulp was placed on squares of paper and treated similarly to curettings, differential counts being made subsequently in small areas here and there where the histology was preserved.

In 50 consecutive cases, excepting those that were discarded because of unsatisfactory preparations, differential counts on 500 cells were done in the areas of maximum cellularity of the marrow from each bone classified as slightly hyperplastic or more. It is to be emphasized that as a result of this selection of areas of maximum cellularity the differential counts apply only to at least fairly well advanced hemopoiesis. Maximow's classification of the cells was selected, the purpose being to obtain a definite anatomical grouping of the cells rather than to adhere to any particular idea of histogenesis of the cells.

In the 100 cases, the presence and absence of red marrow in the long bones occur in orderly combinations. When hyperplasia is present in the tibia, it is also present in the femur and humerus. This combination occurred in 2 cases. It may be present only in the femur and humerus. This distribution of red marrow occurred in 31 cases. Hyperplasia may be present in the humerus alone, which occurred in 25 cases in this series. In the remaining 42 cases no hyperplasia in the long bones occurred.

Seecof in unpublished observations states: "Hyperplasia of the marrow in the long bones does not take place uniformly. It appears first in the humerus, then in the femur, last in the tibia . . .," — and he states further, "Moreover, when recession sets in the hyperplasia disappears first from the tibia, then from the femur and last from the humerus." The data presented are in agreement with Seecof's concept.

The correlation of the distribution of the red marrow in the long bones with age shows that with advancing years there is an increasing tendency for the marrow of the long bones to be fatty. For example, in the fourth decade, 1 case out of a total of 13 showed no hyperplasia in the long bones; in the sixth decade 8 cases out of 20 showed no hyperplasia in the long bones; in the eighth decade 9 cases out of 12 showed no hyperplasia in the long bones. Custer has recently emphasized the decreasing cellularity of the marrow in different bones with advancing years.

The differential counts on the marrow of the different bones in the same case were essentially the same. The maximum variation occurred in the case of the neutrophilic myelocyte, 7 cases showing a variation of over 10 per cent, the maximum being 19 per cent. Therefore, for the series it is fair to represent each case by an average of the percentage values of each cell type in the different bones of the same case. This was done and the data for each cell type were arranged in a percentage frequency table from which the range of average was calculated. For this series of cases the range of average for each cell type is de-

finer as the limits in percentage values within which 80 per cent or more of the cases occur.

The range of average for the neutrophilic myelocytes is from 25 to 45 per cent; that of the segmented cells and metamyelocytes from less than 1 to 6 per cent each, and that of the promyelocytes 1 per cent and less. The arithmetical averages are 32 per cent, 3 per cent, 4 per cent, and 0.5 per cent respectively.

The line of division between the myelocytes and the promyelocytes was arbitrarily drawn between those cells with the cytoplasm more than half filled with the specific granules, and those cells with the cytoplasm less than half filled with the specific granules. Therefore, it is seen that there is a noticeable lack of transition forms, *i.e.* the promyelocytes, between the immature basophilic stem cell, the hemocytoblast and the more mature form, the myelocyte.

The range of average for the polychromatophilic erythroblast and the normoblast is from 5 to 25 per cent each; that of the proerythroblast 1 per cent and less; that of the total number of cells of the erythrocytic series 20 to 45 per cent. The corresponding arithmetical averages are 13 per cent, 17 per cent, 0.3 per cent, and 29 per cent. Here too, there is a noticeable lack of transition forms between the hemocytoblast and the more mature erythroblast.

The range of average of the small lymphocytes is from 5 to 20 per cent, that of the plasma cells from less than 1 to 7 per cent, and that of the medium size lymphocytes 2 per cent and less. The corresponding arithmetical averages are 13 per cent, 4 per cent, and 0.7 per cent.

The extreme limits of dispersion of the hemocytoblasts are from less than 1 per cent to 3 per cent; that of the eosinophilic granulocytes from 1 to 8 per cent; that of the megakaryocyte from less than 1 to 2 per cent; that of the reticulo-endothelial cells from 2 to 8 per cent. The corresponding arithmetical averages are 0.8, 4, 0.5 and 4 per cent.

There was one exception in the case of the eosinophilic granulocytes not included in the data and the classification of the reticulo-endothelial cells is at variance with Maximow's classification. The unclassified cells were 5 per cent and less.

Maximow has described as characteristic of homoplastic hemopoiesis the hemopoietic pattern in which there is a lack of a significant number of transition forms between the hemocytoblast on one hand and the myelocyte and erythroblast on the other, the latter two type cells furnishing the adult granulocytes and erythrocytes of the blood. On this basis hemopoiesis was of the homoplastic type throughout in this series.

The average range of variation of the various type hemopoietic cells in material from routine autopsy has been shown.

It is believed that these data are of value in the study of the pathology of the bone marrow.

Discussion

(Dr. David P. Seecof, Montreal.) I want to congratulate Dr. Williams for undertaking a study of the cytology of the marrow in the different long bones. At this time, since he mentioned the unpublished observations I have made, it might be advisable briefly to point out some of the important practical facts that have been accumulated since I began examining the marrow of more than one long bone at autopsy back in 1920. The need for such an examination became apparent while performing an autopsy on a patient who died during a megaloblastic crisis, in whom during life up to 40 per cent of the circulating red

cells were nucleated. When the tibia was opened, I found that the marrow was completely fatty or resting and the question arose — where did the circulating nucleated red cells come from? The marrow of the femur showed a slight hyperplasia, but in the humerus the marrow was markedly hyperplastic. A search of the literature revealed that it had long been known that the marrow of long bones differed from that of flat and tail bones in mammals and in birds. It appeared from the biological and zoölogical literature that in the *normal* adult the usual demands for hematopoiesis were supplied by activity of the marrow of the flat bones and that the marrow of the long bones did not manufacture red cells unless there was a call for increased hematopoiesis, as in anemic states. I called the attention of Dr. Francis Peabody to this fact and he agreed on the fallacy of taking tibial punctures in studying the condition of the bone marrow in pernicious anemia. Since 1920, examination made on the long bones and the flat bones in over 1000 autopsies led to the following conclusions: If the marrow in the tibia, as also Dr. Williams' data showed, is resting or aplastic it does not mean that there is no hyperplasia in the other long bones. On the other hand, if the tibial marrow is hyperplastic, there is no need for examining the other bones, since they will all show hyperplastic marrow. This is of practical importance in all intravital bone-marrow studies. The fact that the long bones are not normally forming red cells suggests that if an *intra vitam* biopsy is desired for the study of the bone marrow it should be taken from the sternum, because of the more accessible bones; the sternum is always in active hyperplasia, whereas the tibia may or may not be. Similarly, if the problem is to determine if increased activity or hyperplasia of the marrow is present in the body, then the tibia is the bone to be examined. These facts seem to hold for all ages, as Dr. Williams pointed out. I have yet to see a case of aplasia of the marrow of the flat bones. The condition called aplastic anemia, in my opinion, does not exist, because I have seen many cases of pernicious anemia before liver therapy was instituted, *i.e.* before 1924, in which the marrow in the long bones was entirely aplastic and that in the flat bones was hyperplastic. Aplastic anemia was probably often erroneously diagnosed because only the long bones were examined. In old age one rarely finds active hyperplasia in the long bones. However, I have never seen aplastic marrow in the flat bones at any age or in any clinical disease. This holds for agranulocytosis also.

(Dr. William Boyd, Winnipeg.) There is a very striking plate in the book on pernicious anemia by Davidson and Gulland which shows a large number of bones in the body removed at one autopsy. It represents in color the remarkable patchiness of the hyperplasia, and certainly emphasizes the great fallibility of examining only one bone, or only one or two places in a bone.

(Dr. Williams, closing.) I should like to add in closing that I did not mention, because of lack of time, that the remainder of the cell types, which I did not show, were counted, but I did not have time to present the data.

HISTAMINE AND LEUKOCYTOSIS. Virgil H. Moon and (by invitation) Marshall M. Lieber, Philadelphia.

Abstract. The intravenous injection of histamine phosphate into cats is followed by prompt leukocytosis, preceded occasionally by slight leukopenia. The subcutaneous injection of histamine phosphate into monkeys is likewise followed by leukocytosis. Histamine phosphate injected intravenously or subcutaneously in man produces a transient leukopenia followed by a moderate leukocytosis.

This is not so marked as in cats and monkeys. The increase consists chiefly of polymorphonuclear neutrophils. Following single injections the leukocytic count in cats and in man returns to normal in 24 hours; in monkeys the increase frequently persists 48 hours.

Discussion

(Dr. E. M. Medlar, Mt. McGregor.) There is one thing I wish to point out in regard to the leukocytic reaction and that is this — you cannot pay any attention to less than a shift of 50 per cent in the total count, nor less than 10 per cent in the differential count. Conclusions should not be drawn unless there occurs a shift greater than that mentioned. I have taken individuals and punctured their fingers in bed, walking around and at rest, sitting in a chair, at 5 and 10 minute intervals, over $\frac{1}{2}$ to 2 hours, and in those individuals I have found a shift as high as 50 per cent in the total count, and as high as 10 per cent in the differential count. Such a degree of shifting is of no significance except that it shows the leukocytes to be unevenly distributed in the circulating blood.

(Dr. Moon, closing.) The variations were much more marked in cats and in monkeys. The average increase in cats was over 100 per cent, and the increase seen in monkeys ranged between 200 and 1000 per cent. The evidence thus far in human subjects would not be significant, were it not accompanied by this marked evidence seen in the experimental animals. I believe that the experiments indicate another analogy between histamine and Sir Thomas Lewis's H-substance which he investigated in human subjects. I would make a final suggestion that the form in which the histamine is present in the human case is not known, that is, the particular chemical combination in which it exists, and there is a possibility that that combination is more active physiologically than histamine phosphate.

STUDIES ON THE CHEMOTROPIC PROPERTIES OF POLYMORPHONUCLEAR LEUKOCYTES AND LYMPHOCYTES. Harold M. Dixon (by invitation) and Morton McCutcheon, Philadelphia, Pa.

Abstract. Chemotropism of human leukocytes was studied *in vitro* by the following method. A clump of bacteria is placed on a glass slide and dried; a drop of blood from the finger tip is lowered onto the bacteria and allowed to spread between slide and coverslip. This preparation is observed with the microscope at 37° C., and by means of a drawing ocular the path of each leukocyte is recorded at minute intervals. The net approach of leukocyte to bacteria is measured, and this distance is divided by the total path traversed in the same length of time. The quotient is taken as the measure of chemotropism and ranges in value from +1.00, when the leukocyte approaches the bacteria in a straight line, to -1.00 when it moves directly away. Polymorphonuclear leukocytes begin to move as soon as the preparation is warmed. All the cells in the same microscopic field as the bacteria display positive chemotropism, while in more distant fields the intensity of the reaction diminishes with the distance. When *Staphylococcus albus* was used as the source of attraction, no change in chemotropism was found over a 5 hour period; the width of the zone of attraction did not increase. With the highly pathogenic yeast, *Torula histolytica*, polymorphonuclears were at first attracted, but later some cells moved away in a nearly straight line, displaying negative chemotropism. No difference in rate of locomotion was observed between leukocytes that displayed positive chemotropism and those

moving at random in remote fields, that is, we observed no relation between rate of locomotion and chemotropism. In contrast with polymorphonuclears, lymphocytes were neither attracted nor repelled by *Staphylococcus albus* or tubercle bacilli. They were not attracted to caseous material from tuberculous man or chimpanzee. Blood lymphocytes from a case of acute lymphatic leukemia and from a case of infectious mononucleosis were not attracted by *Staphylococcus albus*. We have obtained no evidence that lymphocytes display chemotropism.

Discussion

(Dr. H. Gideon Wells, Chicago.) I should like to ask the relative speed of these different types of cells, lymphocytes and polymorphonuclear leukocytes.

(Dr. E. T. Bell, Minneapolis.) Is there any difference in behavior of the lymphocytes containing a small amount of cytoplasm as compared with those which contain a large amount of cytoplasm?

(Dr. Milton J. Grand, New York.) Were the bacteria grown in agar or in broth?

(Dr. Dixon, closing.) In answer to Dr. Wells' question about the speed of the cells, we found that the lymphocytes moved on an average of 13 microns per minute, while the polymorphonuclear leukocytes moved on an average of 30 to 33 microns per minute.

In reply to the question concerning the amount of cytoplasm of the lymphocytes, we have been unable to see any difference in the behavior of cells with relatively large amounts of cytoplasm and those with relatively small amounts of cytoplasm.

We have used both broth and agar and some of the tubercle bacilli were grown on protein-free medium. Also the organisms were washed in some instances, and still we found no difference in the chemotropic response.

THE MEGAKARYOCYTE IN THE CIRCULATING BLOOD WITH SPECIAL REFERENCE TO HODGKIN'S DISEASE. E. M. Medlar, Mt. McGregor, N. Y.

Abstract. It is generally recognized that the megakaryocyte is found in the circulating blood in various pathological conditions. It has been reported as present in cases of acute lobar pneumonia, myelogenous leukemia, polycythemia vera and in rare instances in Hodgkin's disease. A very rare case of megakaryocytic leukemia has been reported. In all of these reports the presence of fully differentiated megakaryocytes is what has been observed. The chief purpose of this presentation is to show the maturation phenomenon of the megakaryocyte from the marrow stem cell to the fully differentiated mammalian type of megakaryocyte as found in the circulating blood. In this maturation process there is a stage in which it is difficult and at times impossible to distinguish individual megakaryocytes with certainty from monocytes. In fact, the author believes that many cells which have been called monocytes in blood smears are megakaryocytes in the process of maturation. In other words, attention is drawn to the presence of immature megakaryocytes in the circulating blood. They probably occur much more commonly and in more pathological conditions than is at present recognized.

In regard to Hodgkin's disease, the author believes that immature megakaryocytes are a consistent finding in the circulating blood. In his study of blood smears from 20 cases of proved Hodgkin's disease they were present in all but two instances. In 5 cases where the author had an opportunity to examine

blood smears weekly over periods of months these cells have been present consistently. The number varied from 5 to 40 present.

Whether the presence of megakaryocytes may have a diagnostic significance in Hodgkin's disease is at present under investigation. From present data it would seem that if there is to be any diagnostic significance in the finding of megakaryocytes, it will have to be from the percentage and persistence on repeated examinations rather than on single observation.

The main distinguishing features between the monocyte and the megakaryocyte are: (a) the nucleus tends to be more complex and lobulated in the megakaryocyte than in the monocyte; (b), the cytoplasm of the maturing megakaryocyte is more granular and stains deeper with Wright's blood stain than does that of the monocyte; and (c), the immature megakaryocytes tend to have less cytoplasm compared to nuclear volume than do mature monocytes.

Discussion

(Dr. Virgil H. Moon, Philadelphia.) I recall some 20 years ago Bunting and Yates at Madison made blood counts in cases of Hodgkin's disease and described large mononuclear cells to which they did not apply a definite name cytologically, but it occurred in about 5 to 20 per cent in the blood examined in cases of Hodgkin's disease. I should like to ask Dr. Medlar whether in his opinion the megakaryocytes which he has found are identical with the cells described by Bunting and Yates.

(Dr. J. Furth, New York City.) It is to Dr. Medlar's merit that he has focused our attention on the megakaryocytes. This presentation brings up the question of the limitations of morphological studies for the interpretation of cytogenesis. The cytoplasmic fringes interpreted as evidence of ameboid movement are possibly artefacts. Dried blood smears do not seem to be suitable to determine the identity and potentialities of the large mononuclear cells with basophilic cytoplasm. May I ask Dr. Medlar if he has studied these cells in the live state?

(Dr. Medlar, closing.) In reply to Dr. Moon's question, I sent blood smears from some of these cases out to Dr. Bunting and he said they were the same cells he had seen, but he thought they were monocytes and they had their origin in the bone. I agree that they come from the bone marrow and that they are monocytic in type, but I believe they are really megakaryocytes.

In reply to Dr. Furth's questions, I myself have not made any study of these cells in life. I may say in the case I showed which was diagnosed as monocytic leukemia the doctor who had the case studied these cells very carefully in supravital preparations and he found these cells moving all over the field, so there is no question in that case but that the cells are distinctly ameboid.

As to their being artefacts, all I can say is that I have been as careful as I could be in selecting cells for demonstration to rule out as far as possible artefacts in the smear.

BONE CHANGES IN LEUKEMIA: PATHOLOGICAL FINDINGS. I. H. Erb, Toronto, Canada.

Abstract. The changes in bone in acute leukemia in childhood may be grouped under the following headings: (1) infiltration; (2) rarefaction; (3) proliferation; (4) degeneration, and (5) hemorrhage. These changes are illustrated in two

cases of leukemia, one that of a girl who died at the age of 6 years, the other that of a boy who died at the age of $2\frac{1}{2}$ years.

Infiltration by leukemic cells may occur in bone as it does in the liver and kidneys or elsewhere and may involve the marrow cavity, haversian canals or subperiosteal regions. By replacing bone marrow it may give rise to profound anemia. It is not visible in roentgenograms. Rarefaction occurs chiefly toward the ends of the long bones, but may occur anywhere along the shaft and involve both cancellous and compact bone. Proliferation of new bone occurs underneath the periosteum following stripping up of the periosteum by infiltrating leukemic cells. This new bone formation, as well as the areas of rarefaction, are demonstrable by roentgenograms. Degeneration of masses of leukemic cells, as well as hemorrhage into the marrow cavity, may occur in the course of the disease, but are of little clinical significance.

BONE MARROW IN AGRANULOCYTOSIS. R. P. Custer, Philadelphia, Pa.

Abstract. Study of the bone marrow of 12 cases of idiopathic agranulocytosis showed a striking qualitative likeness. Clinically the cases presented either acute progressive or chronic continuous profound neutropenia, relative lymphocytosis (actual lymphopenia), no anemia or thrombocytopenia of consequence, and no hemorrhagic phenomena. Necrotizing mucosal lesions were more or less prominent. The uniform findings were as follows:

(a) Marked proliferation of myeloblasts which does not proceed at the expense of the other marrow elements and never results in the so-called "replacement anemia."

(b) Failure of these cells to mature, resulting in paucity of myelocytes and practically complete absence of segmented forms.

(c) Normal or slightly increased red blood cell formation.

(d) Slight hyperplasia of otherwise normal megakaryocytes.

(e) Infiltration of lymphocytes and plasmocytes with formation of folliculoid aggregations of these cells.

Degeneration and relative hypoplasia of the marrow were noted in 2 cases, although qualitative changes were similar to those in the other 10. Comparison of differential marrow counts from idiopathic and secondary agranulocytosis (arsphenamin and septic neutropenia) showed marked dissimilarity in that maturation of granulocytes was complete in the secondary types.

The following table attempts to link the various causes of neutropenia *per se* with changes in the marrow to afford the clinician better opportunity for classification of cases as idiopathic or symptomatic agranulocytosis:

I. With relatively "full" marrow, as result of:

(a) Severe toxemia (usually bacteria), through primary stimulation of granulopoietic tissue, then destruction of cells *in situ* or on entry into the circulating blood.

(b) The leukoses (leukemias), *viz.*:

(1) Aleukemic myelosis, through overproduction of granulocytes that either do not leave the marrow or are destroyed on entering blood.

(2) Lymphadenosis, through replacement of granulopoietic tissue.

(3) Reticulosis, through replacement of granulopoietic tissue.

(c) Idiopathic agranulocytosis (agranulocytosis of Schultz, agranulocytic angina, malignant neutropenia), through defective maturation of myeloblasts (most cases show full marrow; see II (e)).

II. With relatively "*empty*" marrow, as result of:

- (a) Severe toxemia (usually chemical), sometimes specific for neutrophils (benzol).
- (b) Marrow exhaustion, through protracted anemia, toxemia or infection.
- (c) Aplastic anemia (idiopathic), congenital or acquired.
- (d) Irradiation (roentgen ray or radium).
- (e) Idiopathic agranulocytosis (the occasional case).

The presence of a lesion of maturation specifically confined to the granulopoietic series, not reduplicated by diseases of known etiology, entitles idiopathic agranulocytosis to a place as a disease entity. Regarding the relation of amidopyrin it can hardly be deemed more than an exciting factor, certainly not the only one; the disease may prove to be a proliferative allergic condition with several precipitating agents.

Discussion

(Dr. E. T. Bell, Minneapolis.) I should like to ask Dr. Custer if he can give us the proportion of myeloblasts in the marrow of untreated pernicious anemia; also if these differential counts were made on smear preparations of marrow.

(Dr. David Seecof, Montreal.) I should like to ask if the same long bone was studied in all the cases. As I pointed out previously (in discussing Dr. Williams' paper), it would be unfair to make comparisons otherwise. Incidentally, the last table of Dr. Custer's data again raises the important question in relation to studies on the circulating blood and marrow changes, namely, what correlation is there between the findings in the circulating blood and the findings in the marrow of the bones? I found it is possible to have abnormal circulating blood findings in relation to myelocytes and erythroblasts and yet have the marrow of the flat bones yield negative findings, or that the marrow in long bones be resting or aplastic. At a given moment there may be no parallelism between the findings in the blood and in the marrow. I have worked for many years trying to find out what the time relations were between the appearance of abnormal circulating blood conditions and the appearance of abnormalities in the cytology of the bone marrow. I think in view of the fact that there is no correlation between the findings in the circulating blood in regard to the red cells and the myelocytes, at a given moment (or immediately before death), and the marrow in the long bones in particular, that it would be better to study the flat bones such as the vertebrae, sternum, ribs, and possibly the skull bones (where we find activity of the marrow all the time). The changes seen in the long bones may be those which have just been initiated. It seems to me that for these special cytological studies the marrow of the flat bones should be examined.

(Dr. J. Furth, New York City.) A characteristic feature of agranulocytosis is that it is not associated with anemia and as the studies of Dr. Custer show, erythropoiesis of the marrow is not disturbed. In the differentiation of acute leukemia from agranulocytosis, would it not be better to examine "reserve" marrow, *e.g.*, the marrow of the femur or tibia? Common agranulocytosis is regarded by Dr. Custer as an "idiopathic" disease different from that produced by known chemicals. Since recent studies have shown that long continued administration of chemicals such as amidopyrin produces similar changes in the marrow, is it not conceivable that the primary process is essentially the same in both conditions, namely, injury to the marrow, which fails to put out leukocytes in response to inflammatory irritants?

(Dr. George Shanks, Toronto.) I should like to call attention to the fact that there are a great many points of resemblance between kala azar in which there is a visible parasitic blocking in the hematopoietic system everywhere, and granulocytopenia with an active bone marrow. It seems to me that the study of kala azar, intensively, would shed a good deal of light not only on these problems but on the process of hematopoiesis.

(Dr. Custer, closing.) In answer to Dr. Bell's question, I do not recall with sufficient accuracy the differential counts on my pernicious anemia cases to give percentages of myeloblasts; I can only state that they do appear in the marrow in relatively few numbers, compared to megaloblasts. The differential counts were all done on bone marrow sections rather than smears; I believe that smears or imprints do not give nearly as accurate an index of the relative proportion of cells of the different series as does sectioned material. Counts on sections are more difficult, however, in that cytology may not be so clearly demonstrable as in smears, but I think that they can be accurately done.

The counts shown to-day were all made from the femur because I wanted to show the changes of early hyperplasia versus those of late, *i.e.*, possible qualitative differences between fulminating cases and cases of long-standing agranulocytosis. Qualitatively the counts on marrow from flat bones in these cases were very similar to those in the femur; had there been any significant variation, I should have mentioned it.

The point that Dr. Seecof made about early changes in previously fatty marrow differing from those in previously cellular marrow is true in the majority of instances; in other words, we often find hyperplasia in the adult femur to be of a type suitable to the demands of the occasion, if I may be permitted this teleological comment; adult femoral marrow will present predominantly myelocytic change in severe infection, erythroblastic in anemia. There is a reflected hyperplasia, however, in the various cell series not immediately concerned.

With regard to Dr. Furth's comments, I do not believe that the disease should be classed among the chemical toxemias, even though amidopyrin may appear to be one of the exciting causes; there is one qualitative change in the marrow that separates it from the former group, *i.e.*, a failure of maturation of the neutrophils: let us still call it idiopathic agranulocytosis. This maturation defect is not observed in other diseases and I think that we should still regard the condition, tentatively at least, as a disease entity.

POLYCYSTIC KIDNEYS. E. T. Bell, Minneapolis, Minn.

Abstract. Polycystic kidneys are found once in about every 500 postmortems, and from 5 to 10 per cent are unilateral. In our autopsy service about one-third of the cases occurred in infants, the majority of which were stillborn. There are relatively few clinical cases between infancy and the age of 25 years, but the disease is always congenital.

We may distinguish a surgical type in which the patient presents symptoms and signs referable to one kidney, *viz.* pain, tumor, hematuria, infection, and so on.

In the medical type the symptoms are those of acute or chronic renal insufficiency, and the functional disturbances correspond to those of contracted kidneys. Attacks of hematuria are, however, distinctive.

Edema is rarely prominent, and cardiac failure is unusual. The systolic blood pressure is 150 mm. Hg or higher in over 50 per cent of the cases that have been

reported and hypertension is somewhat more frequent in advanced than in early stages of the disease. Cardiac hypertrophy often develops but is much less pronounced than in primary hypertension. Retinal changes of the hypertensive type may be found especially in those with very high blood pressure. Some patients live many years after symptoms have developed. When the renal reserve is low, *i.e.* in advanced cases, pregnancy causes a typical nephritic toxemia, but there is no disturbance when the renal reserve is good.

There is abundant evidence that polycystic renal disease has a strong hereditary tendency.

The pyelogram is of great diagnostic value in cases where the diagnosis is otherwise difficult.

In the newborn group the outstanding structural changes are: the presence of numerous cysts, hypoplasia of parenchyma, *i.e.* a great reduction in the number of nephrons, and an excessive amount of interstitial connective tissue.

The numerous "glomerular" cysts are interpreted as vestigial structures derived from the first three or four generations of tubules.

In the subclinical group there is abundant renal parenchyma between the cysts; while in the clinical group the parenchyma may be reduced to a few small scattered islands.

The progressive atrophy of the parenchyma is brought about chiefly by continuous expansion of the cysts. Arterial disease plays a minor rôle in this process except in the occasional case in which true primary hypertension is superimposed on the cystic disease.

The arteries usually show a marked intimal thickening which is attributed chiefly to disuse atrophy but partly to hypertension. Medial fibrosis in the arteries is explainable on the basis of age.

The arterioles show no marked intimal diseases except when primary hypertension is a complication. However, they often show a marked medial fibrosis. This process is not true arteriosclerosis.

Kampmeier's theory of the origin of the cysts is favored.

One case is described (No. 44) in which compensatory dilatation of persistent tubules in a hypertensive contracted kidney caused it to resemble the true congenital cystic kidney.

Discussion

(Dr. George Baehr, New York City.) In the study to which Dr. Bell has very kindly referred, the arterial tree of polycystic kidneys was injected with a barium gelatine mixture and the kidneys then studied roentgenologically. We found that in advanced polycystic kidney disease most of the arterioles become obliterated and impermeable to the injecting fluid. As one would expect in any diffuse disease of the kidney, or any other organ, extensive arteriolar disease had occurred secondarily. Dr. Bell's observations that the vascular alterations are largely a medial disease are extremely interesting. In 1 case we have even encountered a necrotizing arteritis in the arterioles of the polycystic kidney which morphologically was identical with the type of disease described by Fahr and Volhardt as malignant sclerosis. The changes in the arterioles which occur secondarily in polycystic kidney disease must be at least a contributing cause in the production of arterial hypertension, renal insufficiency and ischemic sclerosis of the remaining small areas of renal parenchyma. This arteriolar sclerosis may even be complicated by the process which has been described as malignant sclerosis.

(Dr. Bell, closing.) I am inclined to think that this appearance of malignant sclerosis which Dr. Baehr refers to is an associated disease. I have 1 case in which there is a little arteriolar sclerosis of the ordinary type, and it is apparently a case of primary hypertension combined with polycystic kidney. Arteriolar sclerosis is so rare in polycystic kidneys that I think it is simpler to think of it as a combination of two diseases rather than as an arteriolar disease due to the polycystic kidney itself.

ANOMALIES OF THE CIRCLE OF WILLIS AND SERPENTINE ANEURYSMS OF THE
INTERNAL CAROTID ARTERY: THEIR RELATION TO ENCEPHALOMALACIA AND
CEREBRAL HEMORRHAGE. Otto Saphir, Chicago, Ill.

Abstract. A number of instances of encephalomalacia and cerebral hemorrhage were encountered in which a careful examination revealed the absence of occluding lesions of the vessels at the base of the brain. A morphological explanation for the encephalomalacia was not recognized until the circle of Willis was carefully examined and anomalies found, in some instances with resulting interruption of the circulation between the internal carotid and vertebral arteries. Anomalies of the circle of Willis have been frequently encountered since close attention has been paid to these vessels. In other instances occluding lesions were found in the internal carotid artery either in its petrous portion within the temporal bone or in its cavernous portion. These occluding lesions were the result of severe arteriosclerosis with formation of serpentine aneurysms and with production of ridges which completely occluded the lumen.

An interference with the passage of blood through the circle of Willis or occlusion of one internal carotid artery does not make itself manifest until the circulation through the other internal carotid artery, or both internal carotid and vertebral arteries, respectively, is impaired. The impaired circulation may be caused by an arteriosclerosis of the arteries of the brain or by a failing heart, evidence of which may be deduced from the findings of myocardial fibrosis and chronic passive hyperemia of the various organs. As a result of the impaired vis a tergo and arteriosclerosis of the vessels of the base of the brain, complicated by complete separation of the two arterial channels of the brain or occlusion of one of the internal carotid arteries, encephalomalacia may ensue. Before one resorts to an explanation primarily based on functional disturbances, three factors must be investigated:

1. The entire course of the internal carotid and vertebral arteries must be examined for occluding lesions.
2. The patency of the circle of Willis should be ascertained.
3. Morphological evidence of a failing heart must be sought.

Discussion

(Dr. Shields Warren, Boston.) I should like to ask Dr. Saphir if he considers these aneurysms to be of acquired or congenital origin. I judge from the tendency of association with other anomalies in the circle of Willis that the congenital origin is a very definite possibility. I have had 1 case of marked bilateral aneurysm of the anterior carotid also with absence of the posterior communicating branches in which a long course of neurological symptoms of a rather puzzling sort increasing in intensity suggested that the arterial change had been present from birth.

(Dr. E. Libman, New York City.) I want to make a clinical point in connection with this important communication of Dr. Saphir's, and that is that clinicians are not sufficiently in the habit of examining the abdominal aorta and the carotid arteries for evidence of arterial disease. It was pointed out, a long time ago, that it is possible clinically to detect a carotid occlusion.

Just the other day Dr. Saphir visited me and we had a case in the office of a patient who presented a carotid obstruction due to a rather large plaque in the wall. The artery showed only slight pulsation above and exaggerated pulsation below the obstruction. On the right side there was a smaller plaque, without any evidence of obstruction. In occasional patients such plaques are very tender, and while I do not want to draw any definite conclusion, I wish to add that several times cerebral accidents occurred within 6 months to a year after the observation was made.

(Dr. Norbert Enzer, Milwaukee.) Aneurysms of the type described by Dr. Saphir should not be confused with those of distinctly congenital origin. Seven cases of congenital aneurysm have come to our attention. These always occur in the circle of Willis and its branches, always in multiples, and always at the bifurcation of vessels. These aneurysms are subject to thrombosis or rupture. In either event, the clinical syndrome in the congenital aneurysms is more apt to be referred to the meninges; whereas the aneurysms of the type Dr. Saphir described are associated with deep-seated cerebral symptoms.

(Dr. Saphir, closing.) In our cases I do not believe that the aneurysm can be explained on a congenital basis, but rather because of the tortuosity of the vessels which I think is the result of a severe arteriosclerosis.

As far as Dr. Libman's comment is concerned, I firmly believe that these cases are overlooked not only clinically but also pathologically.

AIR EMBOLISM FOLLOWING INTRAVENOUS DRIP. Kornel L. Terplan and (by invitation) Carl T. Javert, Buffalo, New York.

Abstract. In a colored male, 36 years of age, resection of a jejunal ulcer was performed. Postoperative course was uneventful for 12 days; then nausea and emesis developed. Duodenal decompression and continuous intravenous drip of normal saline and 5 per cent glucose were therefore employed. The drip functioned well for 48 hours but an occasional interruption resulted when the patient removed the needle from the vein. A cannula was then ligated in the cubital vein and in some manner the adapter to the cannula was dislodged. It was estimated that 15 minutes elapsed before it was replaced. During this period no blood ran from the cannula; the intravenous fluid wetted the bedding. On replacing the adapter, the drip continued and 400 cc. of saline were given. Nine hours following the dislodgement of the cannula, the patient became increasingly restless, confused, irrational, dyspneic and pulseless and convulsions developed. He made several attempts to get out of bed. Death occurred 20 hours after the adapter was dislodged.

Autopsy was performed 5 hours after death. The right ventricle and pulmonary artery contained fluid blood on which floated pinkish red clots, foamy, sponge-like in appearance, with distinct gaseous bubbles on their surfaces. These clots floated in water. The right atrium contained cruor clots without air. The foramen ovale was closed. The left ventricle was practically empty. The other findings comprised a huge peptic ulcer in the duodenum with no gross bleeding point; tarry stool in the intestine; no peritonitis; no thrombo-emboli in the lungs.

The brain was anemic and edematous, weighing 1450 gm. Smears and cultures of the air-containing blood clots were negative for bacteria. There was no post-mortem autolysis, and gas bacillus effects could positively be ruled out. Bacteria stains were entirely negative. Smaller branches of the pulmonary artery also showed air bubbles within their bloody content. In the frontal lobe and the island of Reil distinct focal necrosis with complete disappearance of ganglion cells in the third layer was found. These changes resembled the ischemic lesions as described in circulatory disturbances of varied etiology (anoxemia, insulin shock, fat and air embolism).

The unusual spongy appearance of the clots in the right ventricle could be explained only by the presence of air in the ventricle, which was included in the clots when coagulation took place. The clots in the right atrium did not contain air. Apparently this excluded any postmortal entry of air into the blood in the right ventricle. The right ventricle was dilated and flabby; the left ventricle contained neither blood nor clots. Careful gross and histological examination did not reveal any other cause of death.

Attempts to reproduce foamy clots artificially at autopsy by injection of different quantities of air into the jugular vein in cases where fluid blood was suspected were unsuccessful. The air was found in the large veins but no clotting had occurred, although 15 to 30 minutes were permitted to elapse before the heart was removed.

It is believed that air entered the venous circulation when the adapter was dislodged from the cannula. The aspiratory effect of the negative intrathoracic pressure, together with a lowered venous pressure which accompanied the ligation of the vein, were considered as instrumental factors in the entrance of air. It must be remembered that no blood escaped from the cannula when the adapter was out of place.

A GROUP OF CASES CHARACTERIZED BY SYSTEMIC VASCULAR ALTERATIONS AND ASSOCIATED FREQUENTLY WITH LUPUS ERYTHEMATODES AND ENDOCARDITIS. George Baehr, Paul Klemperer and (by invitation) Arthur Schiffrin, New York City.

Abstract. Attention is called to a group of cases which are characterized clinically by irregular fever with a tendency to remissions, by involvement of synovial and serous membranes (arthritis), pericarditis, pleuritis, by depression of bone marrow function (leukopenia, thrombopenia, anemia), and by clinical evidences of vascular alterations in the skin, the kidneys and the other viscera. Twenty-three of the cases presented the skin and mucous membrane lesions of disseminated lupus erythematosus. With rare exceptions, the patients were young females, most commonly in the second and third decade of life. Blood cultures were negative.

The macroscopic changes in the internal organs at autopsy were chiefly located within the heart and kidneys. A terminal lobular pneumonia was often present.

Serous membranes were involved in 17 of the 23 cases of lupus erythematosus, pericarditis being present in 12 cases. In 13 of the 23 cases a coarse verrucous form of endocarditis was found upon the mitral or tricuspid valves. In 5 of these the parietal endocardium also showed lesions which conformed to the original description of Libman-Sacks (atypical verrucous endocarditis). In the 8 other cases there were smaller verrucae on the valves. Nine cases had no endocarditis whatever. Aschoff bodies were not found in 22 hearts which were carefully

studied. The kidneys showed multiple shallow depressions in 2 cases. Anemic infarcts were occasionally found in spleen and kidneys, and several cases showed emboli within pulmonary arteries.

Microscopic examination revealed conspicuous vascular lesions in the finer ramifications of the systemic and sometimes also the pulmonary circulation. They were found in the kidneys in 20 out of 23 cases. In the other organs the incidence of vessel changes was not as high. In 6 cases the vascular lesions were widespread in all the viscera. The skin showed similar vascular alterations.

Histologically, the vessel lesions represent a variety of changes: (1) Simple dilation of capillary beds in certain areas, as in the skin, with blood and serous extravasations. (2) Proliferative lesions of the lining endothelium of capillaries, arterioles and venules, associated with thrombi which often obstruct or occlude the lumen. (3) Degenerative and necrotizing lesions in the wall of such vessels, associated with thrombosis and sometimes with hemorrhage into the adjacent tissues. The severer lesions are especially conspicuous in the capillaries and arterioles of the kidney.

Because all three types were often found in the same case, they may be considered as stages of the same underlying morbid process.

The glomerular changes were especially conspicuous in 18 of the cases. The commonest and most characteristic alteration was a peculiar hyaline thickening of the capillary walls which is striking even in sections stained with hematoxylin-eosin. We have described this as the "wire loop lesion." It was present in 13 cases. Proliferative and thrombotic lesions of glomerular loops were frequent. In 2 cases the glomerular changes were sufficiently extensive to be called a true diffuse glomerulonephritis. In 3 cases the proliferative and necrotic process involved only a segment of a glomerulus, thereby creating a superficial resemblance to the embolic glomerular lesions of subacute bacterial endocarditis.

Isolated vascular lesions of similar appearance may at times be encountered in a careful histological study of persons who have died of any acute or chronic infectious process (Sigmund). Our group of cases is distinguished by the systemic distribution of the vascular lesions in various viscera.

Discussion

(Dr. E. T. Bell, Minneapolis.) I feel fairly certain, Dr. Baehr, that these "wire loop" lesions that you pointed out in the first few slides are thickenings of the capillary basement membrane. That thickening occurs almost constantly in eclampsia, in chronic forms of lipoid nephrosis, and in hypertension, and occasionally in various other toxic diseases, even in pernicious anemia. We have to look at the basement membrane of the capillaries as much as at the endothelial cells in understanding these glomerular changes. I think none of these so-called embolic lesions are embolic in the sense that they are infarctions. I think they are due to bacteria which grow and produce a thrombosis of the capillaries. It was originally the idea of Löhlein himself that these were thromboses. He later changed to the idea of infarction.

(Dr. E. Libman, New York City.) This contribution, to my mind, is a very significant one and has implications of a wide nature, into which I cannot enter today because of lack of time. However, I would like to say a few things, mainly in order to clarify what I meant by characterizing certain examples of endocarditis as "indeterminate." This designation was intended to apply to all the cases in which the etiology was not known, and in which there were no distinctive

clinical or pathological criteria. Furthermore, it was intended to convey the idea that further studies might demonstrate that at least some of them represented an unusual reaction to an already known etiological agent. From this comparatively large collection of cases, Dr. Sacks and I segregated a small number because they had something clinical and pathological in common. These we called atypical verrucous endocarditis. We realized that some of the other cases in the indeterminate classification might be found to belong to this group.

As regards the question of lupus erythematosus, we published 4 cases of atypical verrucous endocarditis, of which 2 had facial lesions. Dr. Gross put on record a fifth case of ours, in which there was a secondary infection by non-hemolytic streptococci (subacute streptococcus endocarditis). This case also had an extensive facial eruption. The then dermatologist to Mount Sinai Hospital, Dr. Hermann Goldenberg, stated that while these eruptions resembled acute lupus erythematosus disseminatus, they differed because atrophy, hyperkeratosis and desquamation were absent. It is, of course, possible that others might have included these eruptions in the category of lupus erythematosus. The etiology of this disease is unknown, so that we may well be dealing with an indeterminate disease, from the dermatological standpoint.

In our original publication some vascular lesions were described. Those demonstrated by Dr. Baehr are of a different order. They are entirely new for the description of the pathology of lupus erythematosus, and we consider them an addition to our knowledge of atypical verrucous endocarditis, and of some other cases in the indeterminate group. It remains for further study to determine whether or not the cases (or some of them) of endocarditis described here today, which do not correspond to what Sacks and I called atypical verrucous endocarditis, really belong in the same category. I understand that all these hearts will be examined by Dr. Gross, who in a remarkable paper published in 1932 described the pathological changes in the heart in atypical verrucous endocarditis and concluded that certain of them are pathognomonic of the condition.

It has been pointed out that there are cases that have vascular lesions and no endocarditis, with or without lupus erythematosus. Some years ago I made the suggestion, and Dr. Baehr has made the same suggestion, that there may exist cases due to the same cause or causes as atypical verrucous endocarditis, which have no endocardial lesions. It is possible that in such conditions the vascular lesions will be the characteristic feature.

The whole subject is of wide importance, not only as regards endocarditis and vascular disease, but also as bearing upon the relationship of dermatology to internal medicine.

(Dr. Benjamin Clawson, Minneapolis.) I should like to ask Dr. Baehr what the blood cultures showed in these cases. I cannot quite understand why this group of cases should be classed as non-bacterial and non-rheumatic. I should also like to ask Dr. Baehr just why the hearts are not classified as acute rheumatic endocarditis. It is true that Aschoff bodies were not found in the myocardium, but nobody who has reported a large series of cases of acute rheumatic endocarditis has found Aschoff nodules in all instances, so I cannot see how you would rule rheumatic endocarditis out. The vegetations in the pictures looked to me like the vegetations in acute rheumatic endocarditis. It is true that they extended down on the chordae tendineae which is sometimes found in cases of acute rheumatic endocarditis. Will you explain to us, Dr. Baehr, just why you decided that these hearts are not acute rheumatic endocarditis or subacute bacterial endocarditis?

(Dr. Paul Klemperer, New York City.) May I answer two questions — the question of Dr. Bell and that of Dr. Clawson? The remarkable thickening of the walls of glomerular loops which we have observed was not seen by us in any of the control material we studied, including lipoid nephrosis. There is no reaction for amyloid and no lipoid within the loops. The peculiar hyalinization of the loops resembles, superficially, the thickening one observes commonly in glomeruli of some cases of arteriosclerosis with particular glomerular involvement. But in our cases there was no fat in these tufts. Furthermore, these were young people without arteriosclerosis. One must remember that these lesions were found in young individuals from 12 to 30 years of age, and not in old individuals. In regard to the question of eclampsia, I must confess my experience with eclampsia is limited. Similar lesions probably occur in eclampsia, which is also a toxic disease. We believe these vascular alterations are of toxic origin.

In reply to Dr. Clawson's question, we have examined the hearts very carefully. I think the paper of Dr. Libman and Dr. Sacks proved the fact that the endocardial lesions which they described are not rheumatic. Such extensive lesions on the parietal endocardium do not occur in rheumatic endocarditis.

In regard to the small verrucous lesions which we observed in 8 of our cases, the question of a possible rheumatic origin was seriously considered. In the second heart which Dr. Baehr showed there is a chronic valvular defect on top of which fresh coarse verrucae are found.

Dr. Gross has carried out extensive histological studies on these hearts. I do not know whether one can rely on the histological examination of the valves alone for the diagnosis of rheumatic endocarditis. One has to take the myocardium into consideration. It must be significant that in the 22 cases in which the heart muscle was studied with great care, no Aschoff bodies or other evidence of rheumatic fever was ever found. For this reason we feel that the endocarditis is not rheumatic. Furthermore, identical vascular and glomerular disease was found in 9 cases in which the endocardium was normal.

In regard to the question of bacteria, in 20 of the cases repeated blood cultures were negative. No bacteria were demonstrable in crushing of the vegetations or in sections.

I agree with Dr. Bell that the loop necroses are not of embolic nature. They are probably caused by local thrombosis. In 1 case we can be certain that they are not embolic because there was no endocarditis on the left side of the heart. In another case there was no endocarditis whatever. For this reason we feel the loop necroses which resemble embolic glomerular lesions are local thrombotic lesions.

(Dr. Baehr, closing.) Twenty-three years ago we studied the Löhlein lesions of subacute bacterial endocarditis in fresh and properly fixed material. In the earliest glomerular lesions we demonstrated masses of bacterial emboli microscopically. The clumps of bacteria had been caught in a glomerular loop, thrombosis had then taken place, occluding the lumen of the loop and ultimately necrosis occurred. The embolic origin of the glomerular lesions of subacute bacterial endocarditis was confirmed by Fahr and accepted by Löhlein. The true embolic glomerular lesions of subacute bacterial endocarditis differ in one essential respect from the glomerular loop necroses which we observed in 3 or 4 of our cases of lupus erythematosus. In embolic glomerular lesions, the remaining non-embolized portion of the glomerulus is absolutely normal. In the condition which we are now reporting, the lesion is quite different. Although the necrosis of a glomerular loop may look superficially like the embolic lesion, the rest of

the glomerulus is always altered to a considerable extent. Also no bacteria can be demonstrated in the lesions.

The questions concerning the non-rheumatic nature of the endocarditis have been answered by Dr. Klemperer as well as can be done in these few minutes. The verrucae are free of bacteria and thirty-six blood cultures in 20 cases proved to be negative. Furthermore, the intensity and the widespread distribution of the vascular lesions in various viscera stamp this condition as something quite distinctive. These vascular lesions undoubtedly bear an important relation to the disease which the dermatologists have known for a good many years as lupus erythematosus disseminatus.

ARTERIOLAR CHANGES IN ESSENTIAL HYPERTENSION. Alan R. Moritz and (by invitation) Mary Ruth Oldt, Cleveland.

Abstract. This investigation consisted of a histological study of the walls and measurements of the internal and external diameters of over 10,000 arterioles and small arteries, supplemented by a study of serial sections of selected vessels from the skeletal muscle and gastro-intestinal tract of 38 control and 38 hypertensive individuals.

Thickening of the walls and expansion of the external diameters were characteristic of the hypertensives as a group, but these dimensional changes were not great enough in samples of 75 vessels to permit distinction between control and hypertensive individuals in 80 per cent of the cases. Over 80 per cent of the hypertensive and control cases could be recognized as such by the presence or absence of arteriolar sclerosis in an objective microscopic examination of one section of skeletal muscle without measurements.

The vascular disease in individuals with persistent hypertension appeared to begin in the smallest arteries and not only affected different arteries in the same tissue with varying severity, but affected varying segments of the same arteriole differently. Smooth muscle hyperplasia and medial degeneration characterized the process which progressed with increasing intensity from the larger to the smaller arteries. The primary change in the arteries appeared to be smooth muscle hyperplasia with superimposed segmental medial degeneration. The occasional finding of a dilated, thin-walled, degenerate artery indicated that hyperplasia was not invariably antecedent.

Discussion

(Dr. H. Gideon Wells, Chicago.) I should like to ask what the significance is of these measurements of the lumen of arteries after death. The fact that the lumen is practically the same in sclerotic and normal arteries would seem to me to indicate that the arteries contract as much as they can, no matter in what condition the walls are, and the difference is close to zero. We know from injections of arteries under normal mean systolic pressure that an artery may present a smooth lumen for a long distance, in spite of the fact that in that area are very extensive sclerotic plaques. The lumen is the same, where the sclerotic plaques are, and where they are not. I do not see the significance of these internal diameter measurements after death.

(Dr. E. T. Bell, Minneapolis.) This is an interesting line of study which Dr. Moritz has taken up. The difficulties, as he no doubt realizes, are very great, since we do not know accurately the changes that occur after death. The younger the person, the greater the contraction after the vessel is taken out of the

body. In a young person the contraction of a large artery is sometimes as much as 30 per cent after it is removed from the body. How much they contract in a piece of muscle we do not know, but they probably contract more in younger people than in older ones who have stiff arteries. Another thing which causes a variation is that there is a difference in the number of hours after death at which the tissues are fixed. I have found that in a few hours after death the lumen is much smaller than after 48 hours. There is a rigor in the muscle that passes off after 48 hours. These changes introduce factors which are difficult to control.

(Dr. Moritz, closing.) In reply to Dr. Wells I wish to say that we did not assume that measurements of lumen diameters gave absolute information as to the patency of vessels in life. It was necessary, however, to measure lumen diameters to determine the relative wall thickness of vessels. The fact that the walls of the smallest arteries were thickened in the hypertensives was regarded as significant. Although this thickening was a group characteristic for the hypertensives it was not useful in distinguishing between hypertensive and non-hypertensive individuals.

In reply to Dr. Bell it can be said that there was no significant variation in the relative thickness of arteriolar walls of the control group that could be related to age. The work just reported was preceded by experiments designed to show whether physiological states of vessels, rigor or varying technical methods in the preparation of tissues would affect the relative thickness in arteriolar walls. No significant effect could be related to any of these factors so far as comparing the mean wall to lumen ratio of vessels in one sample with another was concerned.

THE PATHOLOGY OF ADENOMA OF THE BRONCHUS. Coleman B. Rabin and Sylvan Moolten (by invitation), New York City.

Abstract. Since the report of 12 cases of polypoid adenoma of the bronchus by Wessler and Rabin in 1932, 9 additional cases have been observed at the Mount Sinai Hospital. These are now presented together with a pathological study of the original 12 cases for the following reasons:

1. The tumors are still considered to be extremely rare because only sporadic cases have been reported.
2. They present difficulty in histological diagnosis which has resulted in their being reported erroneously as malignant tumors. In other cases their benignity has been recognized on clinical grounds, but the pathologist has been unable to differentiate them from malignant tumors.
3. Definite pathological criteria are presented by which these adenomas may be differentiated from carcinoma.

Discussion

(Dr. William Boyd, Winnipeg.) The remarkably long clinical duration is characteristic of these cases. In a recent case of mine the patient had severe periodic attacks of hemoptysis for 24 years. The microscopic picture was much more benign in type than most of those we have seen on the screen to-day.

STUDIES ON THE MITOSIS RATE IN TUMORS OF SEVERAL MAMMALIAN SPECIES. Albert E. Casey, University, Va.

Abstract. A dependable method having a low coefficient of error was employed to determine the average number of mitoses per 1000 tumor cells in some 300 tumors of man, mouse, rat, rabbit and dog. The tumors included a wide assort-

ment of benign and malignant tumors of both connective and epithelial tissue types, and such conditions as Hodgkin's disease, lymphatic leukemia, and so on. The average rate of mitosis was 10 per 1000 for the 300 tumors with variations from 0 to 32 per 1000. In some 30 tumors the rate of mitosis in the metastases was compared with the rate in the primary tumor and found to be identical in every instance. In 15 of 16 recurrences averaging 1 year after removal of the primary tumor, the rate was also identical. In the heavily irradiated exception the rate was much higher than in the primary tumor. Tumors of laboratory animals were found to have the same range of variation (0-32) as the tumors of man. The transplantable tumors used had rates of mitosis of more than 12 per 1000 and were nearly all anaplastic in appearance, thus corresponding to the more malignant tumors of man. No malignant tumors except basal cell epithelioma (which averaged 2 per 1000) had rates of mitosis of less than 4 per 1000 and no benign tumor a rate greater than 4 per 1000. There seems to be a very sharp line of cleavage between benign and malignant tumors at about 4 per 1000. Tumors from young individuals or from the internal organs averaged a higher rate of mitosis than the tumors from the surface of the body or from old people. Sarcoma and carcinoma of the same grade had similar rates of mitosis.

Mitosis counts were made on a series of about 100 tumors upon which five year follow-ups were available. These included mixed tumors of the parotid, tumors of the breast and cervix, and a few sarcomas. The 25 individuals with tumor mitosis rates of 3 or less were living at the end of the 5 year period, whereas 80 per cent of the 40 individuals with tumor mitosis rates above 12 per 1000 were dead at the end of 5 years. Fifty-seven per cent of the remainder having rates between 4 and 12 were dead at the end of 5 years, the tumors of this group falling into the pathological grading of I and II. The tumors with rates of 12 to 32 correspond to grades II plus, III, and IV. When the mortality was plotted on the ordinate and the mitosis rate on the abscissa with a scale having ascending intervals of 1, 2, 4, 8, 16, 32, a symmetrical smooth S-shaped curve with a sharp ascent at 4 to 7 mitoses per 1000 resulted. This indicates a very high correlation between the rate of cell division in a tumor and its malignancy. The method should prove valuable in studies on the biology of tumors particularly in indicating the relative action of various agents on the constitution of the host or on the growth rate of the tumor cells. The method has seemed helpful in differentiating benign from malignant tumors; it very largely eliminates the present subjective error in the estimation of the number of mitoses and substitutes a mathematically accurate estimate of the growth rate in a given tumor, which growth rate usually remains constant through primary, metastatic and perhaps also the recurrent phases of tumor growth in a given individual.

Discussion

(Dr. Shields Warren, Boston.) Last week Dr. Casey was good enough to tell me about this and I had the opportunity to have one of my men run over these counts on various types of cells. While we all recognize that mitotic activity is roughly parallel to growth rate, I think it is a mistake to depend too much on it, and I was rather interested that two of the relatively high mitotic counts, in the region of 19 and 21, were on tumors of fairly low malignancy, and that in two basal cell carcinomas the counts were 11 and 13 per thousand respectively. In carcinomas of the breast, on the other hand, the count was around 7 and 8 per thousand, as Dr. Casey's was. I feel that the number of mitoses is only one of

many factors that have to be taken into consideration in activity and growth, and particularly in the prognosis of a tumor.

THE EFFECT OF TESTICULAR EXTRACT ON A TRANSPLANTABLE EPITHELIAL TUMOR OF RABBITS. Thomas T. Walker, Watertown, New York.

Abstract. Testicular extract was found markedly to increase the growth of the Brown-Pierce carcinoma of rabbits as compared to control tumors resulting from equal inoculations of the same material. In a series of 13 animals constituting three separate experiments, tumors in the skin and testicle averaged about 200 per cent greater than the corresponding controls. Testicle extract was administered intravenously, 1 cc. per day, starting in some instances after definite tumor growths had appeared. In one experiment it was also injected with the tumor cell suspension. Tumors resulted from each inoculation in the treated series and in one of the controls the tumor grew for a time and later completely regressed. The testicle extract used was prepared from rabbit testicles as described by Duran-Reynals.

THE SO-CALLED BRENNER'S TUMOR OF THE OVARY. Stanley P. Reimann and (by invitation) Clark E. Brown, Philadelphia, Pa.

Abstract. A mixed Brenner tumor of the ovary occurring in a 52 year old woman was recorded. Emphasis was laid upon the biological and morphological differences between these tumors, which have had no notable endocrine activity, and the granulosa cell tumors of the ovary. The inadvisability of the term folliculoma, with which they have been designated previously, was mentioned. Brenner tumors have been discussed in the German literature at some length: Plaut, 9 cases, Meyer, 21 cases. The latter has pointed out their frequent coincidence in the same mass with pseudomucinous cystomas and has shown actual communication between the indifferent epithelium of the Brenner type and cysts lined with typical pseudomucinous epithelium. An etiological relation between the two has been suggested by Meyer's demonstration of pseudomucin-like cells in the strands of Brenner epithelium. The origin of these tumors is thought to be ovarian germinal epithelium in some postovogenic phase of its development, either as congenital foci of Walthard, or as later invasions of the epithelium into ovarian substance.

Discussion

(Dr. Harry C. Schmeisser, Memphis.) Dr. J. M. Maury and I reported a case of bilateral ovarian tumors of the Brenner type in the *American Journal of Obstetrics and Gynecology*, 1934, 27, 290-293. Dr. Robert Meyer of Berlin, who is an authority on this tumor, confirmed the diagnosis. The solid type of Brenner's tumor occurred in the right ovary, which was converted into a very firm, irregular, bluish white translucent mass with a smooth and glistening surface resembling the ovary in shape. It measured 9 by 6 by 3 cm., and weighed 100 gm. On sectioning the mass the knife met with considerable resistance. The cut surface consisted of firm, bluish white fibers enclosing small areas of pink tissue, and was everywhere translucent and very firm. Microscopically the tumor was composed of ovarian type of stroma, rich in cells and fibrous tissue with nests of epithelial cells, characteristic of the Brenner tumor. The pseudomucinous cystoma type of Brenner's tumor occurred in the left ovary, which was converted into a round fluctuating mass with an intact outer membrane whose

surface was smooth and glistening. The mass measured 20 cm. in diameter and weighed 3000 gm. On sectioning the mass it was found to consist of one large cavity with many smaller cysts projecting from the inner surface of its thick, bluish white, translucent fibrous wall. The large and smaller cysts were filled with a mucoid material and lined by a pink membrane, mostly smooth but in a few places mass-like. Microscopically the cyst was lined by pseudomucin secreting columnar epithelium. In the stroma of the wall were epithelial nests and strands of the Brenner type. Both tumors were considered benign. This was apparently the first recorded bilateral case.

(Dr. Alfred Plaut, New York City.) As to the rarity of the Brenner tumor, probably the same rule applies as to many other rare conditions: once it has been demonstrated convincingly it will be found more frequently. In fact, the number of case reports has increased since Robert Meyer's publication. Robert Meyer has observed 4 cases in 20 years in his enormous material in Berlin. In Budapest 5 cases have been seen among 1100 ovarian tumors, and we have seen eight Brenner tumors within 9 years in New York City. When I first saw these tumors I had a disagreement with the tumor authorities in New York because they insisted that these nodules were malignant while I considered them benign. Today we know they are benign. Whether there is a potential malignancy I do not know, but so far no case has turned malignant. There is a statement in the literature that one Brenner tumor in Kermauner's collection in Vienna showed something like malignancy. But this case represents only a metastasis of another carcinoma into that part of an ovary which incidentally is surrounding a Brenner tumor. When I saw a low power photomicrograph of this case in the Handbook of Gynecology I wrote a letter to the Kermauner Clinic in Vienna asking if this were not perhaps a Brenner tumor. Dr. Schiller was kind enough to look the case up and he found it to be a Brenner tumor. At the same time, 2 other cases from Kermauner's collection, which had been put on record as metastatic carcinoma in the ovary, also have been classified as Brenner tumors. Bilateral Brenner tumors must be very rare. Did you find solid tumors in both ovaries?

(Dr. Schmeisser.) No, solid on one and the other side was cystic, and Brenner cells were found in the cyst.

(Dr. Plaut.) Schiffmann has reported a Brenner tumor in one ovary, with Brenner tumor found microscopically in the other ovary. The Brenner tumor in an unusually large percentage of cases is found together with other ovarian tumors, also with rare ones. It has been found together not only with carcinoma of the ovary, but also with granulosa cell tumors and with ovarian struma. Brenner was not the first one to describe this tumor. Eight years before him, Orthmann described the tumor very well and gave good illustrations. Neither he nor Brenner has interpreted the tumors correctly. It was Robert Meyer in Berlin who gave the first correct interpretation.

I am calling the tumor "fibroepithelioma mucinosum benignum," thinking that this name describes the most important features of the Brenner tumor.

Within the last 2 years I have found only one additional Brenner tumor in the material of the Beth Israel Hospital.

PAPILLARY CYSTADENOMA LYMPHOMATOSUM OF THE PAROTID GLAND (ONKO-CYTOMA). David A. Wood, San Francisco, Calif.

Abstract. Cystadenomas of the salivary glands comprise an unusually typical but rare group of tumors. To date 31 authenticated cases confirmed by histo-

logical examination have appeared in the literature. Extensive speculation as to their obscure origin has led to much confusion in nomenclature. For example, we find such terms as onkocytoma, adenolymphoma, branchiogenic adenoma, "orbital inclusion" cystadenomas, and papillary cystadenoma lymphomatosum.

Clinically and pathologically these tumors are slowly growing, benign, occur chiefly in males beyond 40 years of age, and show a characteristic structure except for cystic, tubular, and papillary variations. Papillary cystadenoma lymphomatosum is a descriptive name and refers to the intimate admixture of epithelial and lymphadenoid components which are characteristic. Their lining walls are characteristic in that they are composed of a double layer of peculiar, tall columnar epithelium, the cells of which are swollen, granular, possess distally placed nuclei, and show no cilia. Intercellular secretion capillaries are fairly numerous. The lymphadenoid stroma contains what appears to be definite germinal centers.

Three additional cases are reported, all occurring in males, 37, 48 and 71 years of age. The tumors were all approximately the same size, averaging 5 by 4 by 3.5 cm. They were similar in structure except for papillary and cystic variations. The three tumors were sharply encapsulated and circumscribed. Two were found in the right parotid gland, one in the left.

In support of the thesis that papillary cystadenoma lymphomatosum is a true salivary tumor, another case is presented in which a parotid gland from a cadaver showed a cystic dilatation of an excretory duct. The duct was partially lined by a double layer of columnar epithelial cells supported upon a lymphadenoid stroma presenting a picture strikingly similar to that seen in the 3 cases reported. These peculiar epithelial cells known as "onkocytes" can be found in the salivary glands of progressively aging individuals beyond the age of 20 years. Their occurrence is always associated with the development of a lymphadenoid stroma. The similarity between "onkocytes" and the cells characteristically found in the tumors under discussion is so striking as to suggest the possible adoption of the name "onkocytoma."

THE EFFECT OF PITUITARECTOMY ON THE NATURAL RESISTANCE OF ADULT ALBINO RATS TO HISTAMINE POISONING. David Perla and (by invitation) S. H. Rosen, New York City.

Abstract. The natural resistance to histamine was depressed in completely pituitarectomized rats 1 to 10 weeks after operation. The MLD was one-fifth to one-third that for normal rats.

This decrease in resistance was associated with hemorrhage into or atrophy of the inner zones of the cortex of the adrenals.

Rats in which the posterior lobe and most of the anterior lobe were removed showed a similar drop in resistance. In these instances atrophic changes in the suprarenal cortex occurred. Where a large fragment of anterior lobe remained there was no depression of resistance to histamine and the suprarenal glands were normal.

The repeated injections of large amounts of suprarenal cortical hormone raised the natural resistance of totally pituitarectomized adult rats to histamine poisoning. In some instances the resistance was raised almost to the level of normal rats.

The drop in natural resistance to histamine following pituitarectomy in the rat is probably secondary to the atrophic changes of the suprarenal cortex induced by the withdrawal of the adrenotropic hormone of the anterior lobe.

Discussion

(Dr. David Seecof, Montreal.) I should like to ask how these quantitative determinations were made. Was the lethal dose of the histamine determined by noting whether a rat succumbed to 100 mg., or to 1000 mg., or was the dose repeated daily, or every few hours; in other words, how was the exact quantity determined for the lethal dose of histamine?

(Dr. Perla, closing.) The lethal dose was determined only by groups of rats. A series of rats was taken in which the hypophysis was completely removed; some were given one dose, and others a larger dose, and in that way the range was established. In some instances a rat was used more than once, but the interval between was generally several weeks. The minimum lethal dose could not be determined with a drug such as histamine on an individual animal, but simply the range of a number of animals receiving the same operative procedure.

PITUITARY BASOPHILISM. H. M. Zimmerman, New Haven, Conn.

Abstract. The case is described of a white male, aged 44 years, who presented the clinical features of Cushing's syndrome of pituitary basophilism. The patient came to autopsy as the result of a ruptured dissecting aortic aneurysm and cervical cellulitis. Postmortem examination revealed an inactive basophilic adenoma of the anterior pituitary, cortical hyperplasia and adenomas of the adrenal glands, an adenoma of one of the parathyroid bodies, and diffuse skeletal demineralization.

Discussion

(Dr. William Boyd, Winnipeg.) Was there any calcification in the kidneys? Could the adenoma be recognized with the naked eye? Was it clearly demarcated by a capsule from the surrounding tissue?

(Dr. H. Edward MacMahon, Boston.) I did not understand what kind of an aneurysm was present. I should be interested in knowing if this were a dissecting aneurysm because of the frequency of this lesion in malignant hypertension and of the association of the latter with functioning basophilic adenomas of the adenohypophysis.

(Dr. Zimmerman, closing.) It was a dissecting aneurysm.

In answer to Dr. Boyd's questions — there was no calcification in the kidney. The arteries and arterioles in the kidney appeared normal.

The tumor could not be seen with the naked eye, and we escaped losing it by not stripping the dural envelope of the pituitary. Frequently it has been our custom to remove the dural envelope to facilitate sectioning, and it is possible to tear away the adenomas as they occur beneath the dura, and frequently, as in this case, invade the dural envelope. The tumor was sharply demarcated. It was outlined by a thin, connective tissue capsule, and it had compressed the surrounding pituitary cells so that it did represent an actual adenoma.

A HISTOPATHOLOGICAL STUDY OF ONE HUNDRED HYPOPHYSES. E. M. Butt (by invitation) and Roy M. Van Wart, Los Angeles, Calif.

Abstract. In a study of 126 hypophyses from unselected cases of all ages, an attempt was made to correlate the histological findings with clinical and pathological changes. The hypophyses were serially sectioned on a horizontal plane at 7 microns, every tenth section being mounted and stained with hematoxylin and eosin. From 50 to 90 sections were examined for each hypophysis.

On account of the brief period of time allotted, the presentation was limited to the subject of infiltration of the pars nervosa by epithelial elements, presumably arising from the pars intermedia. Epithelial infiltration of the posterior lobe was found in 83.6 per cent of all cases. The greatest incidence was noted in the age group from 61 to 70. However, good examples of cellular infiltration of the pars nervosa were found in individuals under 30 years of age. No correlation was established between hypertensive states, atherosclerosis or eclampsia and posterior lobe basophilia.

Discussion

(Dr. Alfred Plaut, New York City.) We have in the last 2 years undertaken a similar study comprising about 80 hypophyses. We again are unable to find any relation between the amount of epithelial infiltration of the posterior lobe and the blood pressure. In the course of other hypophysis studies we have (many years ago) attempted to find a correlation between this epithelial invasion and any other condition. We found none. I should like to ask Dr. Butt how many different levels of these hypophyses were examined.

(Dr. Butt.) Serial sections were cut at 7 microns. Every tenth section was examined.

(Dr. Plaut.) Since this was so, I am astonished that you found such a high percentage of hypophyses without infiltration, because if one examines a large number of sections, one finds infiltration practically always in adults, and in children also.

(Dr. David Seecof, Montreal.) I might mention casually some observations on about 2000 pituitaries not sectioned serially, but on taking one section through the middle of the gland. The infiltration of the posterior lobe was found very often and no correlation with any clinical or pathological condition could be found.

(Dr. William Boyd, Winnipeg.) My own experience agrees entirely with the speakers.

(Dr. Butt.) In closing I might say that some 84 per cent had epithelial infiltration of basophilic type, and only 17 per cent were without it. I assume if we had studied every fifth section we would have found a higher percentage of infiltration in all ages.

MORPHOLOGICAL EVIDENCE OF THE EFFECT OF IODINE AND DESICCATED THYROID ON THE ANTERIOR PITUITARY. David Marine and (by invitation) S. H. Rosen and C. Spark, New York City.

Abstract. It has been known for a century that the anterior pituitary is markedly enlarged in individuals with endemic goiter and for more than 50 years that removal of the thyroid is followed by hypertrophy of the anterior pituitary and disappearance of the acidophilic granules. Our experiments on rabbits have shown that iodine administered to rabbits with hyperplastic goiter and hypertrophic anterior pituitary causes a restoration of the acidophilic granules and a great shrinkage of the volume of the gland amounting to a return to normal size and staining reaction.

Iodine administered to thyroidectomized rabbits neither prevents the occurrence of hypertrophy nor restores the acidophilic granules. Desiccated thyroid or thyroxine will prevent the hypertrophy and restore the acidophilic granules and reduce the volume of the gland to normal if hypertrophy has occurred.

These studies indicate that the thyroid secretion exerts as remarkable a controlling influence on the anterior pituitary as the anterior pituitary hormone (thyrotropic) does on the thyroid.

Discussion

(Dr. Isolde T. Zeckwer, Philadelphia.) In some experiments which we have been carrying out the pituitary of thyroidectomized rats was tested for its thyrotropic hormone potency by injection into guinea pigs. The thyroidectomized rat's pituitary at a stage when it is almost devoid of acidophiles has a very high thyrotropic hormone content, greater than that of normal rat pituitary controls. I believe this is direct evidence that the acidophile can be excluded as the producer of the thyrotropic hormone.

(Dr. Marine, closing.) We have not tried rats. Dr. Rosen has tried several such experiments on the rabbit pituitary and we could not find any evidence that the thyrotropic factor was increased in thyroidectomized rabbits, in spite of the fact that after thyroidectomy there is other evidence to believe that the thyrotropic hormone is formed in much greater amounts. Our opinion is that it is not being stored. Others have reported similar findings for the dog and rat. Aaron has shown an increase in the thyrotropic factor in the peripheral blood of thyroidectomized animals and several laboratories have observed the increase in acidophilic granules and in thyrotropic potency of pituitaries following iodine administration to animals with intact thyroids.

READ BY TITLE

MASSIVE LEFT AURICLE. L. F. Bishop, Jr., and (by invitation) Andrew Babey, New York City.

A STUDY OF BACTERIAL CAPSULES WITH SPECIAL REFERENCE TO THE MODIFIED USE OF INDIA INK. E. M. Butt (by invitation), Los Angeles, Calif.

THE EFFECT OF X-RAY ON ENCEPHALITIS LETHARGICA. S. A. Goldberg, M. Brodie and (by invitation) P. Stanley, Newark, N. J.

EXPERIMENTAL SUBACUTE TULAREMIA IN RABBITS. R. D. Lillie and (by invitation) E. Francis, Washington, D. C.

FURTHER STUDIES ON THE MEASUREMENTS OF THE MACRONUCLEOLUS OF CANCER. William Carpenter MacCarty, Rochester, Minn.

BACTERIOPHAGE SERVICE TO ONE HUNDRED STAPHYLOCOCCUS SEPTICEMIAS. Ward J. MacNeal and (by invitation) Frances C. Frisbee, New York City.

COMPLEMENT FIXATION AS A DIAGNOSTIC TEST IN BLASTOMYCOSIS. Donald S. Martin (by invitation), Durham, N. C.

ABSORPTION OF INHALED PROTEINS FROM THE UPPER RESPIRATORY PASSAGES INTO THE BLOOD STREAM. Bret Ratner, New York City.

FAMILIAL BONE ABNORMALITIES IN THE RABBIT. Paul D. Rosahn, New York City.

SUBMAXILLARY GLAND DISEASE OF THE MOUSE. Juanita Thompson, New York City.

THE AMERICAN JOURNAL OF PATHOLOGY

VOLUME XI

NOVEMBER, 1935

NUMBER 6

STUDIES ON THE RELATION BETWEEN MICROGLIA, HISTIOCYTES AND MONOCYTES *

HENRY S. DUNNING, M.D.,** AND JACOB FURTH, M.D.

(From the Department of Pathology, Cornell University Medical College, New York City)

Ameboid phagocytic cells with the ability to store large amounts of colloidal substances are present in most of the tissues and in the blood of animals and man. In the central nervous system,¹ in the retina¹ and in ganglia² they are known as microglia, in other tissues as histiocytes,³ and in the blood as monocytes.³ A survey of the literature indicates that these three types of cells are closely related, and the studies described in this report bring further evidence of their intimate relationship.

Del Rio-Hortega¹ discovered that the phagocytic cells in the nervous system could be stained selectively with silver carbonate. In normal nervous tissue these cells exist as independent elements and are distinguished by their irregular, hyperchromatic nuclei and cytoplasmic processes on which there are thorn-like projections. Following injury to nervous tissue they increase in number, engulf cellular debris and red blood corpuscles, and fat droplets accumulate in their cytoplasm. During this phenomenon the processes become less conspicuous and the cells are transformed into round elements often called compound granular corpuscles. On the basis of the selective and distinctive staining of these cells with silver carbonate Hortega defined them as the third element of nervous tissue and named them microglia. At the present time Hortega's method is the only reliable means of their identification. The relation of microglia to histiocytes and monocytes became evident when Russell⁴ demon-

* Received for publication July 15, 1935.

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strated their ability to store trypan blue, and Lebowich⁵ has recently found bacteria in microglia cells.

Costero^{6, 7} cultivated ameboid cells from the brains of chickens, mice, rats, rabbits, guinea pigs and human embryos and concluded that they were microglia on the basis of their morphological properties in tissue cultures stained by Hortega's method and on their functional property of phagocytosis. He pointed out that the chief characteristics of microglia *in vitro* were the same as those manifested by histiocytes and monocytes cultivated by other workers. Wells and Carmichael⁸ studied the ameboid cells of explants of the brain, periosteum and limb bud of chick embryos, using Hortega's method of staining and supravital dyes; they stated that "the striking resemblance between the cells in the brain cultures and the wandering cells in the cultures of periosteum and limb bud suggested that these two types were identical." Belezky⁹ demonstrated that histiocytes in the mesenchyme of the fowl embryo are morphologically similar to the microglia seen in injured nervous tissue and maintained that histiocytes enter the nervous system along the branches of choroidal and pial blood vessels, whence they migrate into the nervous tissue and develop into microglia. Von Santha and Juba¹⁰ found cells which they believed were primitive microglia near small blood vessels in the brain of a rat fetus weighing 15 mg. They observed that these cells first appeared in the brain near the first vascular channels and maintained that microglia are transformed "blood elements" that have migrated into the nervous tissue from the lumen of blood vessels. The finding by von Santha and Juba of microglia-like cells in the optic bulb outside of the retina and in the non-nervous tissue between the otocyst and the pharyngeal cleft is additional evidence that microglia are not peculiar to the nervous system. Subsequently, Juba^{11, 12} found microglia in the brain of a human fetus 23 mm. in total length and in the brain of a fowl embryo 5 days old. Dunning and Stevenson,¹³ using Hortega's method, stained cells in the normal liver, spleen and kidney of the rabbit, which, on the basis of morphological properties, reaction following injury to these organs and ability to store trypan blue, they concluded were identical with the microglia; their studies of these cells in the spleen indicated that they were histiocytes. Von Mihalik¹⁴ recently concluded that there was no difference between the macrophages in cultures of nervous tissue, liver and subcutaneous tissue of the chicken.

The extensive literature pertaining to the nature of histiocytes has been reviewed by Aschoff,¹⁵ Foot¹⁶ and Maximow.³ Illustrations of histiocytes, referred to as polyblasts, having nuclei and cytoplasmic processes similar to microglia are found in the plates accompanying the comprehensive studies of the elements of the connective tissues by Maximow¹⁷ (Plate I, Figs. 30-35, Plate IV, Fig. 3, and Plate VIII, Fig. 5). Ranvier¹⁸ in 1900 had already illustrated histiocytes, which he called clasmatocytes, in the omentum and mesentery of various amphibia and of mammals, fixed in osmic acid and stained with methyl violet. The nuclei of these cells are similar in shape to those of microglia, but the thorn-like projections on the cytoplasmic processes, so distinctive of microglia, are not evident. The clasmatocytes, he thought, were emigrated leukocytes*; the size and shape of their ramifications seemed to depend partly upon the elements of the tissue between which they were situated. He observed that living leukocytes put out prolongations that adhered to the surface of the warm glass chamber containing them and considered this phenomenon as evidence of the transformation of leukocytes into clasmatocytes. He also described the transformation of clasmatocytes into round cells, indistinguishable from leukocytes, following inflammatory irritation of the peritoneum.

When Aschoff and Kiyono¹⁹ demonstrated the ability of large mononuclear leukocytes to store lithium carmine injected into the circulation, it became evident that a well known property of histiocytes is also an attribute of monocytes. In cultures of normal and pathological human lymph nodes Lewis and Webster²⁰ described epithelioid cells and giant cells, which they thought probably arose from or had the same origin as the large wandering cells which appeared in great numbers in almost every culture. Maximow²¹ infected cultures of lymphoid tissue, omentum and connective tissue with tubercle bacilli and concluded that the epithelioid cells and giant cells arose from histiocytes ("polyblasts"). He also stated that lymphocytes transformed into "polyblasts" and sometimes acquired an epithelioid character. A "polyblast" with nucleus and cytoplasmic processes typical of a microglia cell is illustrated in his paper (Plate 1, Fig. 3). Lewis²² reported the formation of macrophages, epithelioid cells and giant cells from mononuclear leukocytes in incu-

* The words *leukocytes* and *cellules lymphatiques* appear to be synonymous in Ranvier's article.

bated blood of the chicken, mouse, guinea pig, dog and of man. Carrel and Ebeling²³ compared cultivated histiocytes with monocytes of the adult chicken and found that "the essential properties of the blood monocyte and the tissue macrophage appear to be identical, as shown by the appearance of the colonies, their action on the medium, the mode of locomotion and the structure of the cells, their rate of growth, their food requirements and their susceptibility to certain toxic substances." The potentiality of histiocytes and monocytes to transform into epithelioid cells and giant cells has been confirmed by Hetherington²⁴ and Hetherington and Pierce²⁵; according to their observations the lymphocytes play no rôle in the formation of macrophages, epithelioid cells and giant cells.

MATERIAL AND METHODS

1. *Study of Fixed Tissues*

Satisfactory preparations were obtained from the brains of chicken embryos 13, 15 and 16 days old, the liver of a chicken embryo 15 days old, peritoneal folds of a young chicken, the brain of a guinea pig embryo weighing 29 gm. and the mesentery of a mature rabbit.

The tissues were stained with silver carbonate, according to the method of del Rio-Hortega²⁶ for the demonstration of microglia, with slight variations.

The entire brain and liver were fixed in Cajal's formol-bromide solution:

Ammonium bromide	20 gm.
Formaldehyde (Merck)	140 cc.
Distilled water	860 cc.

The brain and liver of chicken embryos were fixed for 17 to 20 hours, but the brain of the guinea pig embryo required 41 hours of fixation. They were then heated in fresh formol-bromide to 50°-55° C. (10 minutes) and rinsed in distilled water. Frozen sections 10 to 15 microns thick were cut. They were received in distilled water, transferred to distilled water containing a few drops of strong ammonia water (a few minutes) and washed in 2 changes of distilled water. They were then placed in Hortega's strong silver carbonate solution:

Silver nitrate, 10 % (in redistilled water).....	10 cc.
Sodium carbonate, 5 % (in redistilled water).....	40 cc.
Strong ammonia water, just enough to dissolve the precipitate.	
Solution filtered and stored in an amber glass stoppered bottle.	

Sections of brain of the chicken embryos 15 and 16 days old required 30 seconds in the silver solution, but those of the younger chicken embryo and of the guinea pig embryo and the sections of embryonic chicken liver required a longer period. They were placed in one dish of silver carbonate for 2 minutes and in a second dish for 3 minutes. Silver was reduced in the sections by blowing them about forcibly in a 1 per cent solution of formalin. They were then washed in 2 changes of distilled water, fixed in 5 per cent sodium hyposulphite, washed in 2 changes of distilled water, dehydrated in 95 per cent alcohol and mounted in Canada balsam after clearing in carbol-xylol made up as follows:

Carbolic acid crystals	100 gm.
Creosote	100 cc.
Xylol	800 cc.

Fragments of peritoneum, after fixation in formol-bromide for 17 hours, without subsequent heating, were treated in the same manner as the sections of brain and liver. Requiring the longer period of staining, they were placed in one dish of silver carbonate for 2 minutes and in a second dish for 3 minutes.

2. *Study of Cultures of Tissues and Blood*

Satisfactory cultures were obtained from cerebral hemispheres of chicken embryos 13, 14, 16 and 18 days old, the livers of chicken embryos 14 and 16 days old, a kidney of a chicken embryo 14 days old, a cerebral hemisphere of a guinea pig embryo weighing 29 gm., the liver of a guinea pig embryo weighing 76 gm., the blood of chickens, two young and one mature, and the blood of a young human adult (two series of cultures).

Preparation of Cultures: The tissue was placed in a dish of Tyrode solution, where it was freed from all visible blood vessels and fibrous portions. It was then transferred to a dish of fresh Tyrode solution and cut up into pieces approximately 1 mm. square. Each piece was placed on a coverslip in one drop of plasma obtained from a chicken starved 24 hours; the tissues from the guinea pig embryos were placed in the plasma of the mother, also starved 24 hours. To the plasma was added 2 drops of Tyrode solution containing 1:10 parts of extract of chicken embryos 8 days old. The coverslips, inverted over hollow-ground slides and sealed with paraffin, were incubated at 37.5° C. Subcultures were not made, nor were the cultures washed.

All of the cultures of blood were prepared by clotting the buffy coat with one drop of extract of chicken embryos after the removal of most of the plasma. The buffy coat was placed in a dish of Tyrode solution and cut up into pieces approximately 1 mm. square. After washing with fresh Tyrode solution coverslip preparations were made in the same manner as the tissue explants, using 1 drop of the donor's plasma and 2 drops of Tyrode solution containing 1:10 parts of extract of chicken embryos.

Half of the explants were placed on coverslips coated with carbon by passing them once through the flame of a burning stick of wood. The single layer of carbon particles deposited on the glass did not interfere with the growth or visibility of the cultures.

At frequent intervals some of the preparations were unsealed. One drop of physiological saline containing 1:20,000 parts of neutral red (National Aniline and Chemical Co.) was placed on the plasma clots, the cultures were resealed and examined on a warm stage.

Staining of Cultures: Most of the cultures were stained with silver carbonate. The method used was similar to that described by Wells and Carmichael,⁸ who applied to tissue cultures the method of del Rio-Hortega²⁶ for the demonstration of microglia in fixed tissue. The coverslips with the adherent plasma clots were fixed in Cajal's formol-bromide solution (20 to 24 hours), then heated in fresh formol-bromide to 37.5° C. (1 hour), washed in distilled water (5 minutes), placed in 25 cc. of distilled water containing 2 drops of strong ammonia water (25 minutes) and washed in 2 changes of distilled water (5 minutes in each). The coverslips were placed in Hortega's weak silver carbonate solution, made up by adding 100 cc. of redistilled water to the strong solution, and heated to 37.5° C. (5 to 9 minutes). After dipping them once in distilled water, silver was reduced in the cultures by waving the coverslips to and fro in a 1 per cent solution of formalin. They were then washed in 2 changes of distilled water (5 minutes in each), fixed in 5 per cent sodium hyposulphite (5 minutes), washed in 2 changes of distilled water (5 minutes in each), dehydrated in alcohol (5 minutes in 50 per cent, 5 minutes in 70 per cent, 5 minutes in each of 2 changes of 95 per cent alcohol), cleared in carbol-xylol (5 minutes in each of 2 changes) and mounted in Canada balsam. Some of the cultures were counterstained for fat. After washing out the sodium hyposulphite from the cultures the coverslips were placed in 70 per cent alcohol (15 seconds),

stained in a saturated solution of Sudan III in 70 per cent alcohol (5 minutes), placed in 70 per cent alcohol (a few seconds), washed in distilled water (5 minutes) and mounted in gum-glycerine.

Carbon particles within cultivated cells were satisfactorily demonstrated by staining the cultures with paracarmine. The coverslips with the adherent plasma clots were fixed in 2 per cent formalin in Ringer's solution (at least 1 hour), washed in 2 changes of distilled water (5 minutes in each), placed in 70 per cent alcohol (5 minutes), stained in Mayer's paracarmine (25 minutes), placed in 2.5 per cent glacial acetic acid in 70 per cent alcohol (5 minutes), dehydrated in alcohol (5 minutes in 95 per cent, 5 minutes in each of 2 changes of absolute alcohol), cleared in 2 changes of xylol (5 minutes in each) and mounted in Canada balsam.

EXPERIMENTAL

1. *Study of Fixed Tissues*

Nervous Tissue

Sections of the brains of chicken and guinea pig embryos in the latter half of development, stained with silver carbonate, were studied.

In the brains of the chicken and guinea pig embryos there are many mature microglia cells with fully developed processes (Figs. 1 and 2), such as are present in greater numbers in the nervous system of animals and man after birth. In the chicken embryos there are also swollen microglia cells with fewer processes about capillaries and just beneath the pia and the ependyma. These cells correspond to the primitive forms of microglia demonstrated by Hortega¹ in the nervous system during late embryonic life and shortly after birth.

Non-Nervous Tissue

A peritoneal fold of a young chicken, the mesentery of a mature rabbit and sections of the liver of a chicken embryo 15 days old were studied. The tissues were stained with silver carbonate in the same manner as the sections of brain.

Scattered throughout the peritoneal membrane of the chicken and rabbit there are numerous isolated cells selectively stained brown. They are distinct from the faintly stained fibroblasts and mesothelial cells, between which they lie, and have irregular, hyperchromatic

nuclei and processes characteristic of microglia cells. They appear to be the "clasmatocytes" of Ranvier.¹⁸

Scattered throughout the liver of the chicken embryo there are many cells selectively stained brown and conspicuous against the gray-colored liver cells and connective tissue. They are found in the connective tissue about the portal veins, between the liver cells and partly or entirely within the sinusoids and do not anastomose with each other. Morphologically they are indistinguishable from the microglia of the chicken embryos. A cell with four irregular processes on which there are numerous thorn-like projections, apparently surrounded by liver cells, is illustrated in Figure 3. The majority of the microglia-like cells in the liver are swollen and have fewer processes than the one in this figure and are partly or entirely within the sinusoids. The presence of blood cells in the cytoplasm of many of them indicates their phagocytic ability.

The cell in Figure 4 is situated between liver cells, and one of its two processes extends into a sinusoid; the cell in Figure 6 is entirely within a sinusoid and lies against its wall. It is morphologically distinct from the endothelial cells of the sinusoid, the nuclei of which appear in the picture. The endothelial cells have elongated, oval, black nuclei from which gray cytoplasmic granules extend from each pole. In sections of the liver of another chicken embryo 15 days old the reticulum was distinctly stained according to a method combining the use of silver nitrate, silver carbonate and gold chloride. In these preparations the capsule of the liver and the walls of the large blood vessels appear to be composed of coarse reticulum fibers, which are continuous with very fine threads of reticulum extending along the sinusoids, but the microglia-like cells are not stained by this method which so distinctly demonstrates the reticulum.

This study indicates that in the liver of the chicken in the latter half of embryonic development there are many cells morphologically identical with the microglia. Most of them lie partly or entirely within the sinusoids and, because of their location, their morphological characteristics and their phagocytic ability, are identified as Kupffer cells. A smaller number of the microglia-like cells are extravascular and do not differ from the histiocytes of the peritoneal membrane. Von Kupffer²⁷ maintained that the cells which have been named after him were endothelial cells and formed a syncytium. This view has been almost uniformly accepted, but the more recent

studies of Zimmermann²⁸ indicate that Kupffer cells are distinct from endothelial cells and do not anastomose with each other. Zimmermann also described cells with numerous processes situated between the sinusoids and the liver cells, which he called pericytes and believed to be related to smooth muscle cells. The histiocyte illustrated in Figure 3 of our article is morphologically identical with the "pericytes" of the liver illustrated in Zimmermann's paper (Plate 28, Figs. 188 and 189). The studies of Zimmermann and our own indicate that the Kupffer cells, commonly called reticulo-endothelial cells, are distinct from endothelial cells and, unlike reticular cells, do not anastomose with each other.

2. Study of Cultures of Tissues and Blood

Cultures of Nervous Tissue

Cultures of the brains of chicken and guinea pig embryos in the latter half of development were studied.

Chicken Brain: During the first day of the cultures, delicate straight fibers extended into the medium from the edge of the explants. These structures were first observed *in vitro* by Harrison,²⁹ who identified them as neuraxones. After the first day they were obscured by two types of cells which appeared about the explants — anastomosing cells, indistinguishable from fibroblasts, and large ameboid cells. The cells resembling fibroblasts multiplied by mitosis and eventually formed a tissue about the explants. The large ameboid cells also multiplied by mitosis, displayed no tendency to anastomose with each other and migrated to the edge of the plasma clots.

During the first few days of incubation the large ameboid cells of chicken brain, in cultures stained with silver carbonate, closely resemble the microglia in normal nervous tissue, except for swelling of their cytoplasm and the presence of pseudopodia (Figs. 7, 13 and 14). They have hyperchromatic, irregular nuclei and cytoplasmic processes on which there are thorn-like projections. As the cells migrated away from the explants, fat droplets accumulated in their cytoplasm, their processes became swollen, shorter and fewer, and they became round (Fig. 24). Such cells, often called compound granular corpuscles, are found in great numbers in areas of necrotic nervous tissue. When the large ameboid cells reached the edge of the plasma clots they flattened out against the coverslips and assumed epi-

thelioid forms. In some cultures these epithelioid forms adhered in great numbers to the coverslip over the explant. They were predominant during a late period of the cultures, usually during the second and third week and, although they were often closely packed together, cell outlines could be distinguished. Occasionally they appeared on the coverslips near the explant during an early period, in one culture as early as the third day. Epithelioid cells in cultures stained with silver carbonate (Fig. 9) are large and flat, circular in contour when isolated, and somewhat square when packed closely together. The cytoplasm of some is drawn out into a tapering sharp process. There are few granules in the peripheral portion of the cytoplasm and it is difficult to define the limits of the cells. Centrally, numerous, brown cytoplasmic granules form a conspicuous area at the margin of which there is usually one nucleus, occasionally two. The nuclei of the epithelioid cells are large, oval or round and pale, containing many fine brown granules and a few larger black granules. Fat vacuoles are usually present in the cytoplasm and are situated within or at the periphery of the central area.

The following attributes of the large ameboid cells of the chicken brain were manifest in the living preparations. The projection of pseudopods from any portion of the cytoplasm was common to all forms. A thorn-like projection on one process of the cell in Figure 14 forms the basis of three pseudopods. At the edge of the cytoplasm of the epithelioid forms there was continuous movement of small pseudopods. The large ameboid cells stored neutral red in abundance. In the forms with processes and in the round forms the dye appeared as distinct red granules evenly distributed throughout the cytoplasm. In the epithelioid forms it was confined to the central cytoplasmic area, where it appeared as a circular patch of red granules or, in an occasional cell, as a rosette about a clear, central spot (centriole). In the cultures planted on smoked coverslips carbon particles were found only in the various forms of large ameboid cells. In the forms with processes and in the round forms the carbon particles were scattered throughout the cytoplasm, but in the epithelioid forms they were segregated in the central cytoplasmic area where, in many cells, they formed a rosette (Fig. 25). The large ameboid cells appeared to be very sticky; they adhered tenaciously to the coverslips and to fibers accidentally incorporated in the plasma clots. Costero⁷ demonstrated the ability of cultivated microglia cells to

cling to threads introduced into the plasma clots. After the epithelioid forms became predominant the large ameboid cells rapidly died. They were last seen alive 5 weeks after incubation.

Guinea Pig Brain: During the first two days of the cultures only neuraxones and cells resembling fibroblasts appeared about the explants. The neuraxones grew more luxuriantly than in the cultures of chicken brain. Large ameboid cells were first seen on the third day and, less numerous than in the cultures of chicken brains, they never migrated as far as the edge of the plasma clots, although they survived *in vitro* for approximately the same length of time. During their life in the cultures they exhibited all of the morphological and functional properties of the large ameboid cells of chicken brain.

Cultures of Non-Nervous Tissue

Cultures of liver and kidney of chicken embryos and of the liver of a guinea pig embryo in the latter half of development were studied.

Chicken Liver: During the first day of incubation fibroblasts appeared about the explants. They were more abundant than the similar cells in the brain cultures, multiplied by mitosis and eventually formed a tissue about the explants. A considerable number of small ameboid cells also appeared about the explants on the first day. Identified in stained preparations as polymorphonuclear leukocytes, they migrated far out into the medium and died during the first week of incubation. During the second day large ameboid cells which moved more slowly than the smaller ones appeared about the explants. They multiplied by mitosis and migrated to the edge of the plasma clots.

During the first few days of incubation the large ameboid cells of chicken liver, in cultures stained with silver carbonate (Figs. 15 and 16), are indistinguishable from the large ameboid cells which appeared early in the brain cultures. They have irregular, hyperchromatic nuclei and cytoplasmic processes on which there are thorn-like projections. As they migrated farther away from the explants fat droplets accumulated in their cytoplasm, their processes became swollen, shorter and fewer, and they became round. During the second and third week those at the edge of the plasma clots flattened out against the coverslips and assumed epithelioid forms identical with the corresponding forms in the brain cultures.

The large ameboid cells in the living cultures of chicken liver ex-

hibited all of the attributes of the living, large ameboid cells of the brain. They remained alive in the cultures for approximately the same length of time, engulfed carbon particles, which appeared as a rosette in many of the epithelioid forms, and adhered to the coverslips and to foreign fibers in the plasma clots. The ability of cultivated Kupffer cells to stick to the fibers of lens paper has recently been demonstrated by Rous and Beard.³⁰ As in the brain cultures, neutral red rosettes were observed only in an occasional epithelioid form, never in the round forms and in the forms with processes, in which the dye was always scattered throughout the cytoplasm.

Chicken Kidney: The cultures did not differ from those of chicken liver, except that few or no polymorphonuclear leukocytes appeared about the explants. The large ameboid cells exhibited all of the properties of the large ameboid cells in the liver cultures.

Guinea Pig Liver: During the first day of incubation small and large ameboid cells began to migrate from the explants. The small cells were identified in stained preparations as polymorphonuclear leukocytes. They were about as numerous as those in the cultures of chicken liver, migrated far out into the medium and ceased to move after the first week. The large ameboid cells moved more slowly than the small ones, multiplied by mitosis and were last seen alive 6 weeks after incubation. They were less numerous than in the cultures of chicken tissues and never migrated as far as the edge of the plasma clots.

In cultures of guinea pig liver stained with silver carbonate during the first few days of incubation the large ameboid cells resemble microglia (Fig. 8). They have irregular, hyperchromatic nuclei and cytoplasmic processes on which there are thorn-like projections. Fat droplets accumulated in their cytoplasm and the cells became round. After the third week epithelioid forms appeared on the surface of the coverslips over the explants.

The large ameboid cells of the guinea pig liver in the living cultures exhibited the following properties. They adhered to the coverslips and to foreign fibers in the plasma clots and stored neutral red in the same manner as the corresponding cells in the cultures described above. They engulfed carbon particles. In one culture a single large ameboid cell containing carbon was observed for 6 days, and camera lucida drawings were made at frequent intervals (Fig. 10). It had several processes on the eighth day, gradually became round

and, after the eleventh day, ceased to move. The culture was fixed and stained on the thirteenth day, and no nucleus is visible in this cell. Carbon particles appeared as a rosette in some of the epithelioid forms.

Cultures of Blood

Cultures of the buffy coat of young and mature chickens and of a young human adult were studied.

Chicken Blood: Three hours after incubation the explants were surrounded by a halo, 1 mm. wide, consisting of small ameboid cells. Nine hours later the halo had expanded to 2 mm. and, near the explants, there were a few larger ameboid cells, which moved more slowly than the smaller ones. At the end of the first day the small cells had migrated to the edge of the plasma clots and the larger ones had formed an inner halo about the explants. In stained preparations most of the small cells were identified as polymorphonuclear leukocytes, but the lymphocytes could not be distinguished with certainty from the free nuclei of erythrocytes. After the first week the small cells ceased to move. The large ameboid cells multiplied by mitosis and migrated to the edge of the plasma clots.

During the first few days of incubation the large ameboid cells of chicken blood, in cultures stained with silver carbonate (Figs. 11, 17 and 18), like microglia cells, have irregular, hyperchromatic nuclei and processes with thorn-like projections. Rod-shaped cells (Fig. 17) similar to those in the cultures of chicken brain (Fig. 13) and liver (Fig. 16) are predominant. As the cells migrated away from the explants fat droplets and nuclear debris accumulated in their cytoplasm and they became round and identical with "compound granular corpuscles." Toward the end of the first week, after many of the large ameboid cells had reached the edge of the plasma clots, they flattened out against the coverslips and assumed epithelioid forms indistinguishable from those in all of the cultures described above.

The attributes of the living, large ameboid cells of chicken blood were essentially the same as those of the corresponding cells in the living cultures of tissues. They engulfed carbon particles, which appeared in many of the epithelioid forms as a rosette, and adhered to the coverslips and to extraneous fibers in the plasma clots. In the neutral red preparations the dye appeared as distinct red granules scattered throughout the cytoplasm of all of the forms with processes

and of the majority of the round forms. In approximately one-fifth of the round forms it appeared as a distinct rosette of red granules about the centriole. Neutral red rosettes were never seen in the round forms of large ameboid cells in the cultures of tissues. In the epithelioid forms the dye was confined to the central cytoplasmic area where it appeared as a circular patch of red granules or, in an occasional cell, as a rosette. After the epithelioid forms became predominant the large ameboid cells rapidly died. They were last seen alive 6 weeks after incubation.

Human Blood: Twelve hours after incubation the explants were surrounded by a halo, 2 mm. wide, consisting of ameboid cells. In a culture stained at this time the larger cells can be identified as monocytes, the smaller cells as lymphocytes, and those of intermediate size as polymorphonuclear leukocytes. They are in approximately the same proportion as in the blood stream. The first cells to die in the cultures were the polymorphonuclear leukocytes. In a preparation stained on the fourth day there is pyknosis of the nuclei and disintegration of the cytoplasm of many and, in a culture stained on the sixth day, no granulocytes are seen. The lymphocytes remained alive for a much longer period of time and could be readily identified in the living cultures. They moved about actively in the medium in the same manner as those in the fresh blood of guinea pigs, described by Opie³¹ in 1904. The lymphocytes gradually decreased in number and were last seen actively moving on the twenty-second day. During the entire period of their existence *in vitro* they maintained their original size, and their structure, in preparations stained with silver carbonate, is that of normal lymphocytes. During the course of incubation the monocytes proliferated by mitotic division, gradually became larger and, toward the end of the first week, assumed the characteristics of the large ameboid cells in the cultures of animal blood and tissues described above.

During the second week of incubation the large ameboid cells of human blood, in preparations stained with silver carbonate, resemble microglia cells (Figs. 12, 20, 21 and 22). The predominant forms are rod-shaped (Figs. 20 and 22) and very similar to the rod-shaped forms of microglia, which are so numerous in the brains of humans with paresis (Fig. 19.) Fat droplets and nuclear débris slowly accumulated in the cytoplasm of the large ameboid cells, their processes became swollen and shorter and the cells gradually became round

(Fig. 23) and indistinguishable from "compound granular corpuscles." After the third week the majority of the large ameboid cells assumed epithelioid forms identical with those in the cultures of animal blood and tissues described above.

The living large ameboid cells of human blood exhibited all of the attributes of those of chicken blood. They engulfed carbon particles, which appeared in many of the epithelioid forms as a rosette (Fig. 26). They adhered to the coverslips and to extraneous fibers in the plasma clots and were last seen alive 6 weeks after incubation. Neutral red was stored in the same manner. In the forms with processes and in the majority of the round forms the dye was scattered throughout the cytoplasm, but in about one-fifth of the round forms it appeared as a distinct rosette. In the epithelioid forms it was confined to the central cytoplasmic area, where it appeared as a circular patch of red granules or, in an occasional cell, as a rosette.

In the cultures of human leukocytes it was possible to observe the gradual transformation of the monocytes into large ameboid cells and to identify the polymorphonuclear leukocytes and lymphocytes, which maintained their original size and morphological characteristics until they died. In the cultures of chicken blood, however, the large ameboid cells appeared about the explants very soon after incubation and their origin was not observed. The granulocytes maintained their original size and morphological characteristics until they died. Since no normal monocytes were seen in the cultures, it is evident that they transformed into large ameboid cells. The fate of the lymphocytes of the chicken could not be determined because of the uncertainty of distinguishing them in stained cultures from the free nuclei of erythrocytes.

DISCUSSION

(1) The following observations support the assumption that the large ameboid cells in the cultures of tissues migrated out of the tissues and only a very small number, if any, arose from the blood in the vessels of the explants: (a) After the removal of visible blood vessels the fragments of tissue used for cultivation were washed in Tyrode solution to remove as much blood as possible. (b) In stained sections of the unwashed organs used for incubation there are few, if any, blood cells other than erythrocytes in the capillaries. (c) If the large ameboid cells were transformed monocytes derived from the

blood in the vessels of the explants, polymorphonuclear leukocytes and lymphocytes should have appeared in the cultures of tissues, as they did in all of the cultures of blood, but neither granulocytes nor lymphocytes were seen in the cultures of brain. A considerable number of granulocytes appeared in the liver cultures, but in stained preparations no lymphocytes can be identified among them. The presence of extravascular foci of myelocytes in various stages of maturation seen in the stained sections of the liver of chicken and guinea pig embryos explains the appearance of polymorphonuclear leukocytes in the liver cultures.

(2) It is often stated that fibroblasts may transform into macrophages and macrophages into fibroblasts.³² In none of our cultures was there any evidence of this transformation. It is very common in cultures of tissues to see large ameboid cells and fibroblasts side by side, as illustrated in Figure 61 (Fischer³²) and in Figure 8 of this paper, but in our cultures it has always been possible, both in the living preparations and in those stained with silver carbonate, to distinguish between these two types of cells throughout their existence *in vitro*. Fibroblasts were never seen in our cultures of blood. Some of the rod-shaped cells in the cultures of human blood somewhat resembled isolated fibroblasts (Figs. 12 and 20), but the constant projection of pseudopods from any portion of their cytoplasm, their ability to phagocytize carbon particles and to store large amounts of neutral red served to identify them.

(3) Von Santha and Juba¹⁰ demonstrated that the microglia first appear in the nervous system near the first vascular channels at a very early period of embryonic development and maintained that microglia cells are transformed "blood elements" that have migrated into the nervous tissue from the lumen of blood vessels. Our observation that monocytes in a semisolid medium transformed into cells indistinguishable from microglia suggests that monocytes are the "blood elements" that transform into microglia cells.

SUMMARY

In fixed tissues stained with silver carbonate the histiocytes in the liver of a chicken embryo and in the peritoneal membrane of a chicken and of a rabbit are morphologically identical with the microglia in the brains of chicken and guinea pig embryos.

In cultures of tissues the large ameboid cells of the brains and

livers of chicken and guinea pig embryos and of a kidney of a chicken embryo exhibited the same morphological and functional properties. During an early period of incubation they had cytoplasmic processes and nuclei typical of microglia cells. Fat droplets accumulated in their cytoplasm and the cells became round. During the latter period of their life *in vitro* they transformed into epithelioid cells. They engulfed carbon particles, stored large amounts of neutral red and survived in the cultures for approximately the same length of time.

In cultures of chicken and of human blood the monocytes transformed into large ameboid cells that exhibited all of the morphological and functional properties of the large ameboid cells in the cultures of tissues. Neutral red rosettes were more frequently observed in the large ameboid cells of the blood than in those of the tissues.

CONCLUSIONS

Microglia and histiocytes are morphologically and functionally identical and constitute a single cell type. Monocytes may transform into cells indistinguishable from this type.

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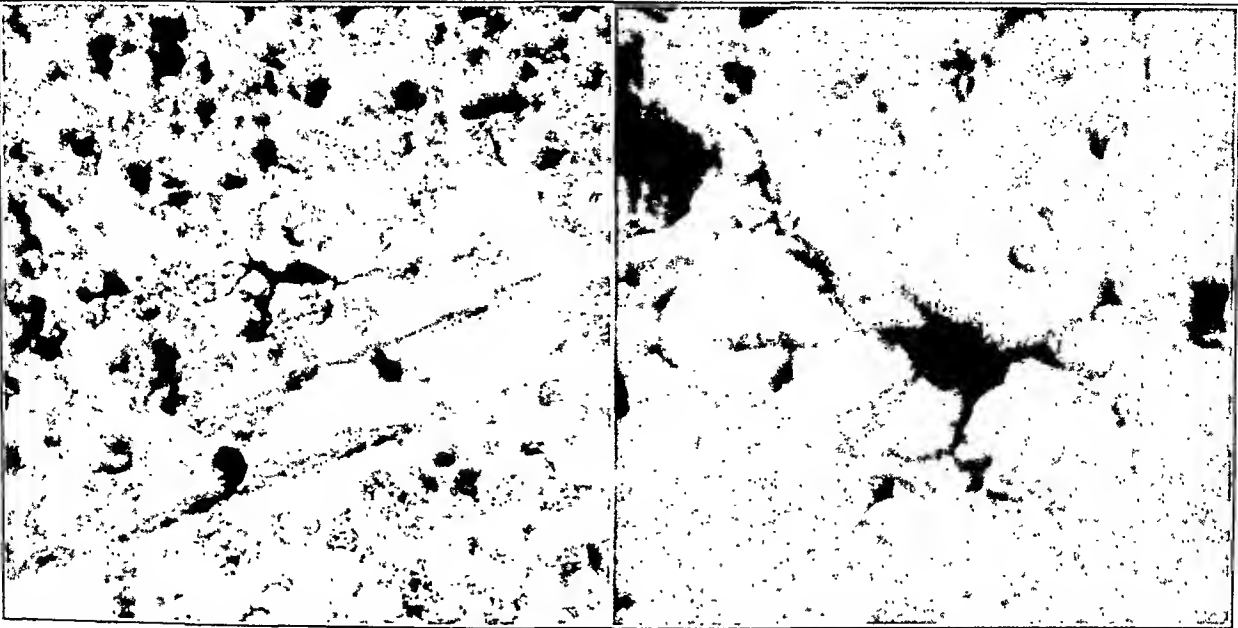
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DESCRIPTION OF PLATES

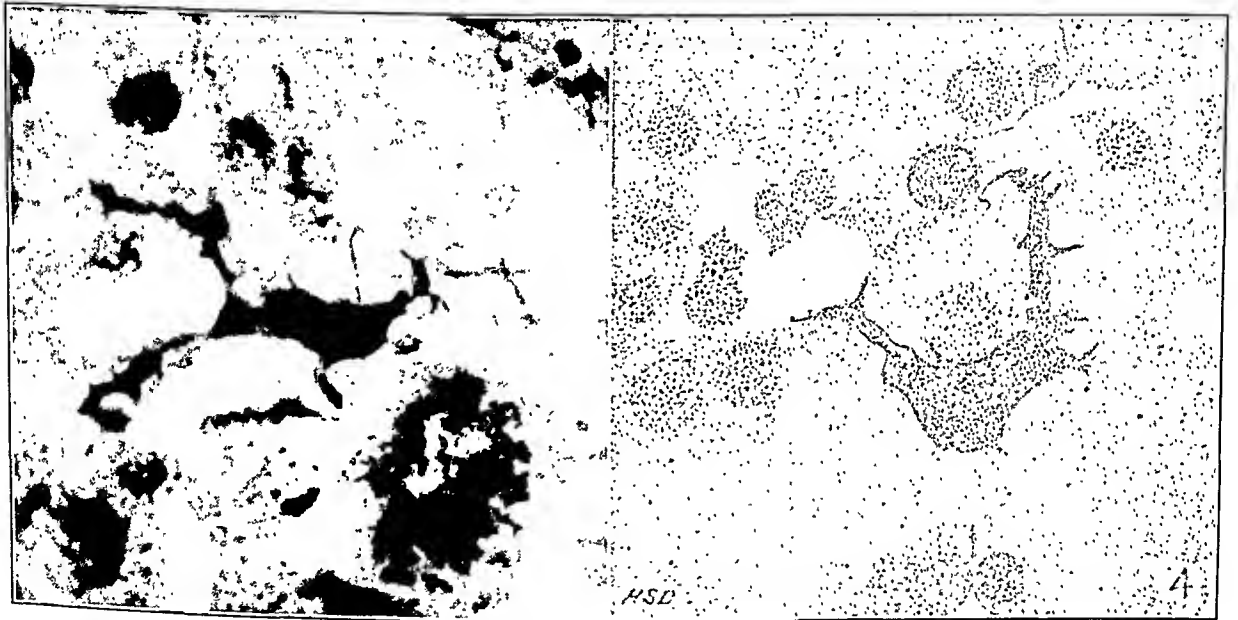
PLATE 118

- FIG. 1. A microglia cell near a capillary in the brain of a chicken embryo 13 days old. Silver carbonate stain. $\times 550$.
- FIG. 2. A microglia cell in the brain of a guinea pig embryo weighing 29 gm. Silver carbonate stain. $\times 1500$.
- FIG. 3. A microglia-like cell in the liver of a chicken embryo 15 days old. Silver carbonate stain. $\times 1500$.
- FIG. 4. A microglia-like cell near a sinusoid in the liver of a chicken embryo 15 days old. Silver carbonate stain. Camera lucida drawing. $\times 2000$.
- FIG. 5. A central vein and radiating sinusoids in the liver of a chicken embryo 15 days old. Silver carbonate stain. $\times 150$.
- FIG. 6. A microglia-like cell (Kupffer cell) and numerous red blood corpuscles in one of the sinusoids included in the square outlined in Fig. 5. Endothelial cells line the central vein and the sinusoids. Silver carbonate stain. Camera lucida drawing. $\times 860$.



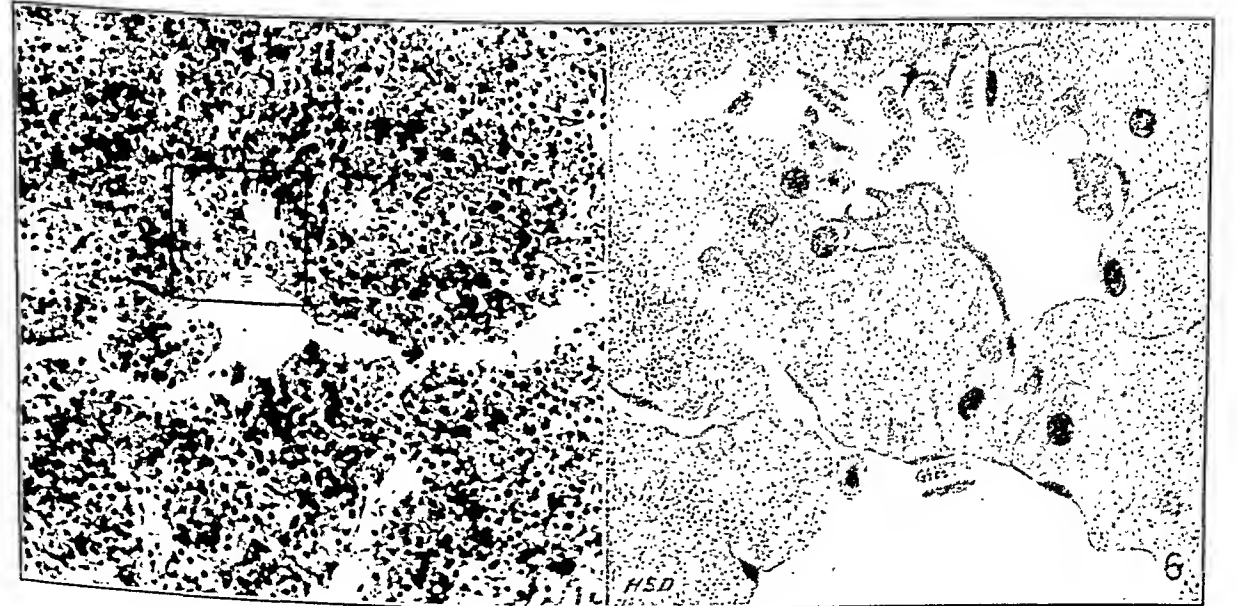
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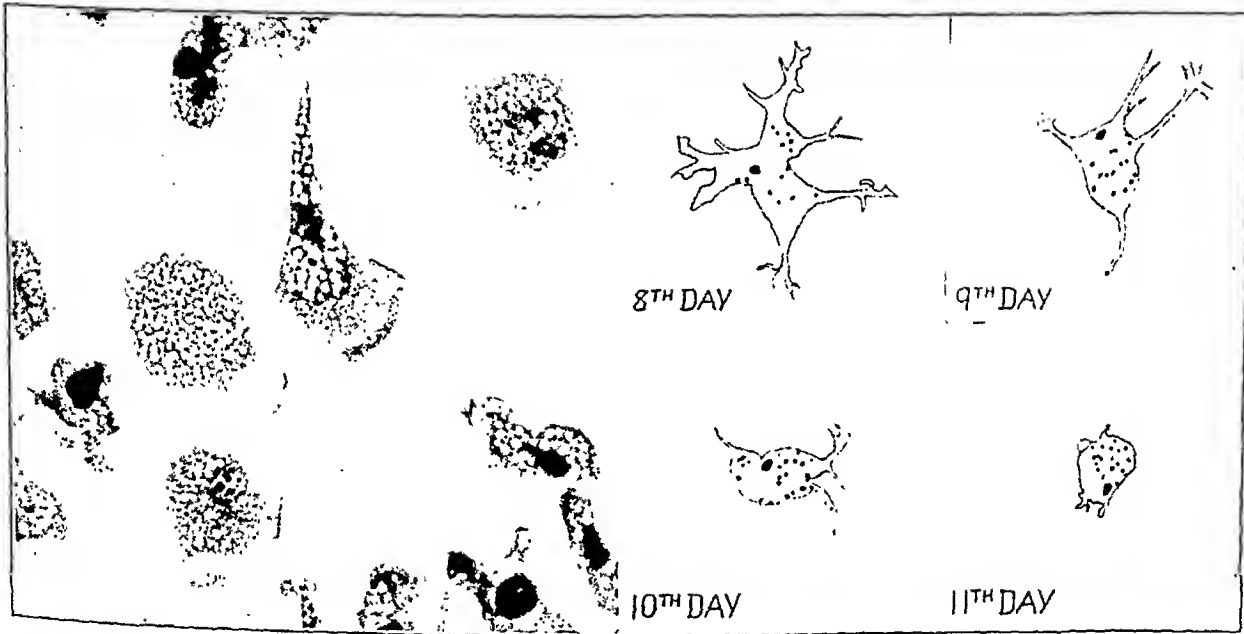
PLATE 119

- FIG. 7. Large ameboid cells with processes near the explant in a 3 day old culture of the brain of a chicken embryo 18 days old. Silver carbonate stain. $\times 100$.
- FIG. 8. Large ameboid cells with processes and fibroblasts near the explant in a 4 day old culture of the liver of a guinea pig embryo weighing 76 gm. Silver carbonate stain. $\times 100$.
- FIG. 9. Epithelioid cells containing fat vacuoles in a 3 day old culture of the brain of a chicken embryo 18 days old. Silver carbonate stain. $\times 600$.
- FIG. 10. Camera lucida drawings of a single large ameboid cell containing carbon particles in a living culture of the liver of a guinea pig embryo weighing 76 gm. $\times 860$.
- FIG. 11. Large ameboid cells with processes in a 4 day old culture of the buffy coat of the blood of a mature chicken. Silver carbonate stain. $\times 100$.
- FIG. 12. Large ameboid cells with processes near the explant in a 7 day old culture of the buffy coat of the blood of a young human adult. Silver carbonate stain. $\times 100$.



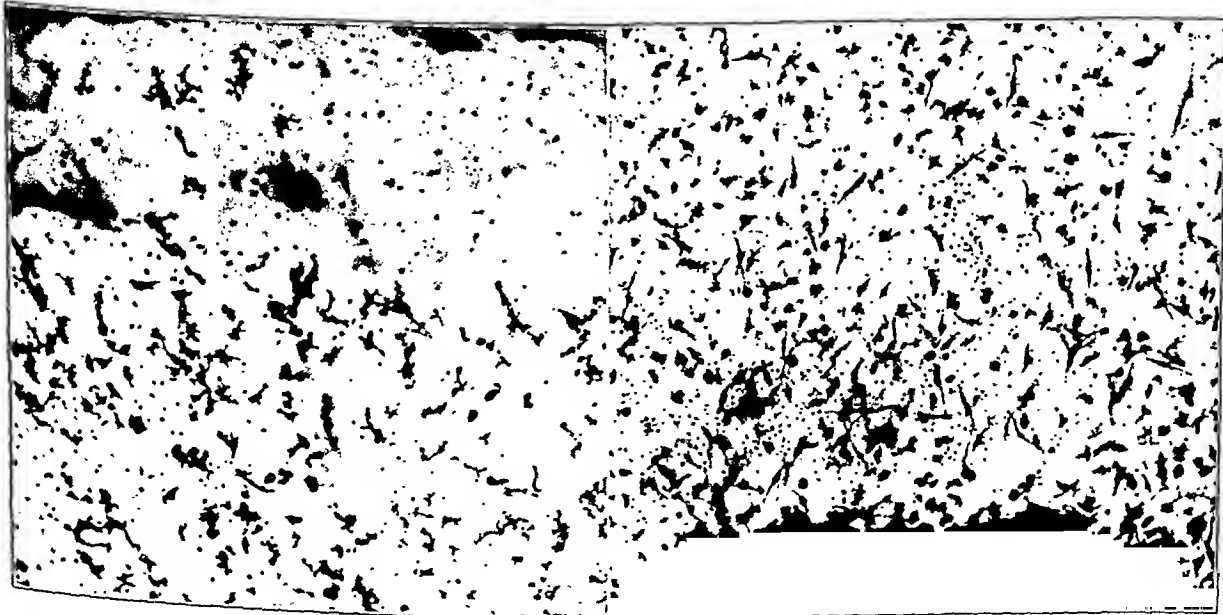
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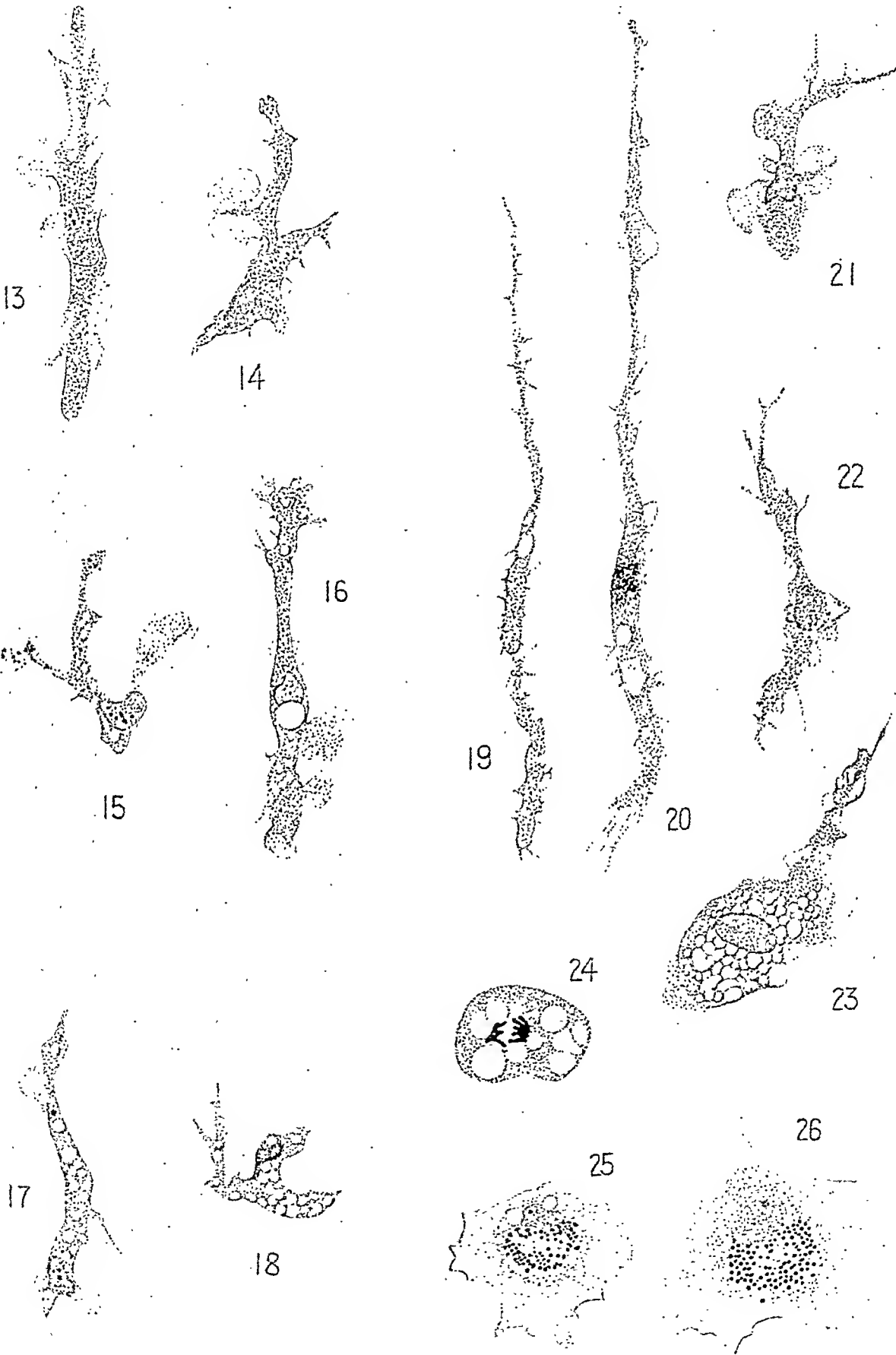
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PLATE 120

Camera lucida drawings of stained cells, at a magnification of 860.

- FIGS. 13 and 14. Large ameboid cells with processes in a 3 day old culture of the brain of a chicken embryo 18 days old. Silver carbonate stain.
- FIGS. 15 and 16. Large ameboid cells with processes in cultures of the liver of a chicken embryo 16 days old. Fig. 15 is from a 4 day old culture; Fig. 16 is from a 6 day old culture. Silver carbonate stain.
- FIGS. 17 and 18. Large ameboid cells with processes in cultures of the buffy coat of the blood of a young chicken. Fig. 17 is from a 2 day old culture; Fig. 18 is from a 1 day old culture. Silver carbonate stain.
- FIG. 19. A rod-shaped microglia cell in a section of the brain of a human with paresis, stained with silver carbonate by Dr. Lewis Stevenson in the laboratory of del Rio-Hortega, Madrid.
- FIGS. 20, 21, 22. Large ameboid cells with processes in a 7 day old culture of the buffy coat of the blood of a young human adult. Silver carbonate stain.
- FIG. 23. A large ameboid cell containing many fat droplets in a 14 day old culture of the buffy coat of the blood of a young human adult, illustrating the transition between forms with processes and round forms. Silver carbonate and Sudan III stains.
- FIG. 24. A large, round ameboid cell filled with fat droplets and exhibiting a mitotic figure in a 6 day old culture of the brain of a chicken embryo 13 days old. Silver carbonate and Sudan III stains.
- FIG. 25. An epithelioid cell containing a rosette of carbon particles in a 10 day old culture of the brain of a chicken embryo 16 days old. Paracarmine stain.
- FIG. 26. An epithelioid cell containing a rosette of carbon particles in a 24 day old culture of the buffy coat of the blood of a young human adult. Paracarmine stain.



HSD

THE CUTANEOUS GLOMUS AND ITS TUMORS—GLOMANGIOMAS *

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For centuries ⁴⁴ a group of small subcutaneous tumors was recognized as the exciting point for severe and often radiating pain. In more recent times these tumors were called "angiosarcoma" in the German literature and "subcutaneous painful tubercle" in various English papers. In 1920, however, Barré ²⁰ clearly defined their clinical manifestations and demonstrated that local excision of the tumor gives complete relief from all symptoms. It remained for Masson to establish the specific histological characteristics of these tumors and to prove their derivation from specialized arteriovenous anastomoses in the stratum reticulare of the cutis. This type of anastomosis is called the cutaneous glomus because of a close analogy to the glomus coccygeum. With their histogenesis established, various names have been given to the tumors. These include glomus tumor, tumeur du glomus neuromyo-artériel, and angiomyoneuroma. Many of these terms are cumbersome. None denotes the fact that the tumors represent an overgrowth of a specific type of arteriovenous anastomosis and form a subdivision of the group of angiomas. If the name were to call attention to this fact, the prognosis and surgical approach, as well as the histogenesis, would be indicated at once. Despite the already too numerous terms applied to the lesion, it seems worth while to suggest *glomangioma* to apply to tumors arising from the cutaneous glomus.

On account of the organoid character of glomangiomas, a description of the normal histology of the glomus will be given and the fate of its various parts will be followed in the tumors.

THE NORMAL CUTANEOUS GLOMUS

A specialized arteriovenous anastomosis, now called the cutaneous glomus, was described first by Hoyer,⁷ but its histological structure was made clear by Sucquet.¹⁴ More recently, important contribu-

* Received for publication May 22, 1935.

tions have been made to the knowledge of the normal glomus by Masson^{11,12} and Popoff.¹³

The glomus is distributed widely but unequally over the cutaneous surface of the body and is by far most frequently encountered on the extremities. It is found most abundantly in the region of the nail bed and at the tips of the digits, while it is present in large numbers on the palmar surface of the first, second and third phalanges of the upper and lower extremities. These anastomoses are demonstrable also on the thenar and hypothenar eminences of the hand and on the sole of the foot near the heel.^{11,12,13} In smaller numbers they are found on the cutaneous surfaces of the upper and lower extremities and in the corpora cavernosa penis and corpus cavernosum urethrae.¹ By a study of serial sections Popoff¹³ found in the great toe of a patient 20 years of age 18 of these arteriovenous anastomoses on the ventral surface, 10 on the lateral surfaces, 24 in the nail bed and 12 in the nail matrix. Grant and Bland⁴ stated that the glomus is much more abundant since they found 593 in the nail bed of the second toe and 296 in the toe pad. They, however, did not use serial sections, which may explain the discrepancy in part. It seems likely that there is considerable anatomical variation in the distribution of the glomus. The diameter of the glomus depends upon its location, those of the pad of the toe measuring 120-220 microns and those of the nail bed 60-150 microns.¹³ The glomus undergoes considerable alteration with age. It is formed imperfectly at birth and reaches its maximum development only in young adulthood. It atrophies in old age even in those individuals in which it escapes arteriosclerotic changes.^{12,13}

The cutaneous glomus occupies a specific zone of the cutis — the stratum reticulare. Its afferent artery arises from arterial branches in the subcutaneous tissue which pursue a course parallel to the surface of the skin. These continue upward and divide into two branches in the stratum reticulare. The larger branch bends at a right angle and continues parallel to the skin while the smaller branch divides again. Some of the divisions of the smaller branch contribute to the formation of arteriovenous anastomoses, while others go to the papillary bodies and the artery itself ends as the artery of the papillary body. The arterial branches constitute the essential parts of the glomus and have been called the Sucquet-Hoyer canals.¹³ The canals pursue an S-like course and have narrow, irregular lumens. A single afferent

glomeric artery forms one to four separate Sucquet-Hoyer canals. When multiple they are situated in contiguity, grouped together by fibrous tissue which, without forming a capsule, isolates them by orientation of the collagenous bundles.¹¹ The endothelial cells are rather large and have oval nuclei. They are placed in two or three rows without an underlying elastic lamina. In the afferent artery at the beginning of the Sucquet-Hoyer canal there are cushion-like endotheliomuscular elevations which, according to Popoff,¹³ serve to direct blood flow. The endothelium is surrounded by a rather thick muscular coat in which indistinct inner longitudinal and outer circular layers have been described,^{11,12} though Popoff¹³ was unable to confirm this finding. Among the muscle cells are large cells with clear or vacuolar cytoplasm which does not contain stainable glycogen, fat or mucin. Their histogenesis is a fundamental problem in an understanding of the glomus since they, more than any other single structure, are peculiar to these anastomoses. These have been called "epithelioid" cells. They may be called *glomus cells* because they are found only in the cutaneous glomus, the glomus coccygeum and their homologues. The name "epithelioid" has been carried on from the time when these structures were thought to be endocrine in nature and should be abandoned. These cells have been described as postembryonal angioblasts,⁸ as modified smooth muscle cells,¹³ and as specialized neuromuscular cells.^{11,12} Their nature, as seen in tumors arising from the glomus, will be treated in the second section of this paper. The glomus cells are in intimate association with a rich network of non-myelinated nerve fibrils which may be demonstrated either by various silver methods¹³ or by Masson's trichrome stain.^{11,12} These can be followed to the larger periglomic nerve trunks. Along the course of the non-myelinated nerves the round and rod-like nuclei of Schwann's sheath are found. These are encountered more frequently as the larger nerve trunks are approached. At the point at which the Sucquet-Hoyer canal begins, there is a distinct collagenous ruffle containing an elastic lamina under the endothelium.¹³ The remainder of the glomus is lacking in elastic tissue, the elastic lamina being resumed at once in the vein of termination of the Sucquet-Hoyer canal. In the meshes of coarse collagenous tissue which surround the glomus there are small arterioles which, arising proximal to the Sucquet-Hoyer canal, supply the clear neuroreticular zone around the canal and the nerve trunks of the peri-

glomeric zone. These preglomeric arterioles do not communicate with the lumen of the Sucquet-Hoyer canal. The arterioles have thin walls with few muscular cells and no neuroreticular elements.¹³ The primary collecting veins are supplied with elastic tissue but present few muscle cells. The collecting veins of the stratum reticulare and stratum subcutaneum are provided with valves, according to Popoff.¹³ The primary collecting veins open into the subpapillary veins and through the latter into deeper veins.

A number of structures have been described which are homologous with the cutaneous glomus. The most important of these is the glomus coccygeum. This structure in the sacral region has been shown by von Schumacher¹⁵ to result from a transformation in an arteriole issuing from the midportion of the sacrum. The cells of the arteriolar walls become larger, clearer, and take on an "epithelioid" appearance. They are in every respect identical with the glomus cells of the cutaneous arteriovenous anastomoses. While the glomus coccygeum appears in young embryos (145-170 mm.),⁸ the cutaneous glomus is absent from the fetus and even premature infants.¹³ The cutaneous glomus is also homologous with the organ of Ruffini¹¹ and the caudal glomeruli of animals.¹⁵

The cutaneous glomus is distributed widely among mammals in much the same regions as in man. Hoyer⁷ described its presence in rabbits, cats and dogs, and its absence in guinea pigs. Grosser^{5,6} pointed out its presence in mice and its absence in reptiles. The glomus is developed to a high degree in birds.¹ It is of particular significance that the glomus has not been described in cold blooded animals and that it is so prominent in birds, whose body temperature in general is above that of mammals.

The function of the glomus was understood very imperfectly until the work of Lewis and Pickering,¹⁰ and Grant and Bland,^{3,4} demonstrated its importance in temperature regulation. They showed that the glomus serves for the maintenance of the temperature of exposed parts and the regulation of loss of heat. By direct observation of the rabbit's ear, they found it difficult to see any anastomoses when a rabbit is kept warm. The anastomoses dilate readily on gentle mechanical stimulation. Even slight stimulation of an area with a rounded glass rod causes local dilatation of vessels and of the anastomoses arising from them. The anastomoses dilate when the nearby skin is pricked. Acetylcholine and adrenalin cause local dilatation of

the arteriovenous anastomoses which is independent of the nervous system. Histamine acts in the same manner as mechanical stimulation. The anastomoses react to nervous stimuli and with weak currents may do so independently of the afferent artery. On contact they dilate vigorously and quickly. To thermal stimuli the reaction is also striking. The ear may be raised to 35 or 40 degrees C. before any great dilatation occurs. However, when the surrounding temperature is lowered below a somewhat variable point (usually about 15 degrees C.), the anastomoses open although other vessels are at first constricted. If the cooling is slight or brief only the anastomoses open. When the animal is warmed again the anastomoses return to normal before the vessels. Grant³ stated that it is mainly through the agency of the glomus that the temperature of the rabbit's ear is maintained when it is exposed to cold. He concluded also that the glomus is an important factor in regulating body temperature of the rabbit, aiding the dispersal of heat by allowing an enormous blood flow through the ear.

Lewis and Pickering¹⁰ showed that in raising the temperature of the room in which the subject is sitting, vasodilatation is produced in the limb in part by a direct effect of temperature on the vessels, and in part by an effect through the central nervous system. The tips of the fingers are usually coldest when the body as a whole is cold, but this warms most rapidly because of the opening of the arteriovenous anastomoses, which are most numerous in the nail bed and finger tips. The blueness of the nail beds in persons exposed to temperatures slightly below those to which they are accustomed is a common observation. In addition, Clara¹ believed those in the penis are associated with the mechanism of erection of that organ.

It is also possible that the cutaneous glomus acts as a shunt in the maintenance of blood pressure. By increasing the passage of blood through the anastomoses the cutaneous capillary bed would be decreased. However, neither Popoff's anatomical studies¹³ nor the physiological observations mentioned above prove this point.

In the present study the pad of the great toe was examined in a comparatively small number of individuals in sections both perpendicular to and parallel with the surface. These were stained with Mallory's phosphotungstic acid hematoxylin or occasionally with hematoxylin-eosin. The arteriovenous anastomoses corresponded in general to the descriptions of Masson^{11,12} and Popoff.¹³ No constant

association of the glomus with such cutaneous structures as sebaceous glands, hair follicles or tactile corpuscles was noted. However, medium sized nerve trunks were found in the periglomic zone adjacent to the outer layers of glomus cells in nearly all cases. Valves were seen in a few veins of the stratum reticulare in the sections, confirming the observation of Popoff.¹³

The pathological changes of the glomus in inflammation, arteriosclerotic gangrene, diabetic gangrene, thrombo-angitis obliterans and supernumerary digits were studied by Popoff,¹³ and those in syringomyelia and old age by Masson.¹¹

GLOMANGIOMAS

In 1924 Masson³³ showed for the first time that certain small cutaneous tumors represent organoid overgrowths of the cutaneous glomus. In the course of the 11 following years, various pathologists confirmed Masson's findings, so that 58 instances of glomus tumor were recorded in the literature. These were obtained from 56 patients, Adair¹⁷ describing a patient presenting 3 glomus tumors of the forearm. The present material consisted of seven lesions, bringing the total number of glomus tumors recorded as such to 65. However, it is possible to find in the earlier literature many descriptions of lesions associated with the syndrome now known to be caused by tumors of the cutaneous glomus. A group of such cases was collected by Chandelux⁴⁴ under the name of "subcutaneous painful tubercle." He pointed out a relation to tactile corpuscles, the presence of numerous dilated blood vessels and the formation of a pseudo-erectile vascular bed. Greig²⁶ reviewed the English literature in this regard and found 20 cases, to which he added 3 of his own, described usually as "painful subcutaneous nodule" but probably representing tumors of the glomus. Kolaczek⁴⁵ in 1878 presented a group of cases under the name of angiosarcoma. Some of these were subungual and produced the symptoms of glomus tumors. Nine years later, Kraske⁴⁶ followed Kolaczek in describing a very painful subungual tumor of the left middle finger as an angiosarcoma. He pointed out that the lack of invasiveness and complete relief by local excision made classification of such tumors in the group of sarcomas somewhat doubtful. As late as 1927, Carstensen⁴³ described an "angiosarcoma" beneath the nail of the right ring finger. The symptoms and microscopic findings were very similar to those

TABLE I
Data on Seven Cases of Glomangioma

Case No.	Age	Location	Symptoms	Relation to trauma	Duration	Gross appearance	Relief by local excision	Adjacent glomus	Nerve trunks in surrounding connective tissue
1.....	yrs. 48	Left upper arm	Local pain on pressure and spontaneously. Radiation of pain to shoulder, left pectoral region and occiput. Described in detail in text	Fol- lowed blow on arm	yrs. 20	Firm, dark red nodule 0.3 cm. in diameter	Immediate and permanent	One in adjacent tissue	Present
2.....	52	Right thigh near groin	Severe local pain on pressure	None	20	Bluish nodule 0.7 cm. in diameter	Immediate and permanent	None	Present
3.....	50	Subungual finger	Local pain on pressure	None	4	Bluish nodule 1 x 0.5 cm.	Immediate and permanent	None	Present
4.....	74	Dorsum of right forearm	Severe and increasing pain, spontaneous or elicited by pressure	None	Many; severe symptoms for 1 year	Elevated, purplish gray tumor 0.7 x 0.5 cm.	Immediate and permanent	None	Present
5.....	79	Posterior aspect right arm 5 cm. above elbow	Local pain on pressure. Contact of clothing sufficient to stimulate pain	None	40	Bluish nodule 0.7 cm. in diameter	Immediate and permanent	None	Present
6.....	57	Lateral chest wall 6 cm. below right axillary pit and 4 cm. posterior to lateral border of pectoralis major muscle	Intermittent spontaneous pain. Pain also elicited by slight pressure	None	18	Soft reddish mass 1 x 0.4 x 0.6 cm.	Immediate and permanent	One in adjacent tissue	Present
7.....	42	Subungual left ring finger	Neuralgic type of pain starting from region of tumor and radiating up arm. Pain elicited by cold or pressure	None	9	Reddish gray nodule 0.5 x 0.4 cm.	Immediate; operation recent	None	Present

of glomus tumors. These are only a few of the references to this type of lesion in the older literature. Because of the lack of complete clinical and histological data in many cases of this type, evaluation of them is difficult and serves no useful purpose. This group emphasizes, however, that the condition is considerably more common than was supposed.

The material upon which the present study was based consisted of 7 specimens, all of which were removed surgically. Blocks from each were fixed promptly in Zenker's fluid. Additional blocks of the tumors in Cases 2, 3 and 7 (Table I) were also fixed in 10 per cent neutral formalin. The material in Zenker's fluid had been used for routine studies but all available fragments were embedded in paraffin and cut in serial sections. Ribbons of three to five sections were placed on slides and stained successively with hematoxylin-eosin, eosin-methylene blue, Mallory's phosphotungstic acid hematoxylin, Foot's modification of Hortega's silver carbonate method for reticulum, Mallory's aniline blue-acid fuchsin-orange G connective tissue stain, Weigert's resorcin-fuchsin elastic tissue stain, and Van Gieson's hematoxylin-picric acid-acid fuchsin. The formalin-fixed material was embedded in celloidin and stained by Cajal's reduced silver method for nerve fibers and Bielschowsky's method for peripheral nerve fibers.

The gross appearance of glomus tumors is rather characteristic. They are always small, those in this series being 1 cm. or less in diameter, while the largest described in the literature is less than 3 cm. in diameter. The tumors vary in color from deep red through shades of purple to blue, the last color being perhaps the most common. The surface is covered by a layer of skin or nail without ulceration or erosion, at least in the 7 instances in the present series. Sharp demarcation from the surrounding tissues is present. The cut surface exudes blood as it is exposed and presents a grayish tint when the blood has been released. Occasionally, as in Case 1, there is a large vessel near the tumor and connected with it.

Upon histological examination, the tumor is found to be composed of contorted vessels with certain peculiarities of their walls which are seen under normal conditions only in the cutaneous glomus and its homologues. Despite considerable variation from case to case and even in the same tumor, as followed in serial sections, vessels can be found which resemble very closely the Sucquet-Hoyer canals of the

normal glomus. As described by Masson,³⁴ and Barré and Masson,²² the vessels of glomus tumors are of two types. In the first of these there are one or two layers of smooth muscle in circular arrangement which are separated from the endothelium by a collagenous membrane. These smooth muscle cells are surrounded in a circular manner by shorter, larger cells with clear or pale staining cytoplasm and a compact globular nucleus. These blend with polygonal glomus ("epithelioid") cells. The second type of vessel presents endothelium bordered by glomus cells without the interposition of smooth muscle fibers. These vessels correspond more nearly to those of the normal Sucquet-Hoyer canal than the previous ones. In the 7 cases of the present series vessels of both types were found in all the tumors, though the relative proportions varied considerably from tumor to tumor and from section to section. Cases 1, 4 and 7 for example, presented only very few vessels of the first type and they might have been missed had the tumor not been examined in serial sections. Some carefully studied cases, however, are composed apparently of but one type of vessel. Mason and Weil³² stated that all the vessels in their tumor were of the second type. As described by Masson³⁴ and as followed in the present material, vessels of the first type are directly continuous with those of the second.

The vessels of glomangiomas usually lack any semblance of elastic laminae. Occasionally, vessels within a glomangioma show well defined elastic layers. These, however, have only smooth muscle cells of the usual type in their walls and do not assume the characteristics of glomic vessels. They may, therefore, be regarded as vessels included in the growth of the tumor or as the preglomic arterioles supplying the glomus from which it arose. The absence of elastic laminae furnishes additional evidence for the derivation of glomangiomas from the Sucquet-Hoyer canals because these canals are lacking in elastic tissue while the preglomic arterioles and postglomic venules are provided with it.

Since none of the glomangiomas in this series showed ulceration, infiltration with any considerable number of inflammatory cells was not encountered. From place to place, basophilic leukocytes were found in rare instances while a few hemosiderin-laden phagocytes were seen occasionally.

About these tumors there are dense collagen fibers, blending with those of the surrounding connective tissue. Usually they resemble

fibers pushed aside in the centrifugal growth of the tumor rather than those of a true capsule. They represent an increase in amount of connective tissue over that seen about the cutaneous glomus normally and the layer is wider only because of the greater size of the contained structure. This is a further demonstration of the lack of tissue destruction caused by glomangiomas.

There is a type of cell which is peculiar to the cutaneous glomus, its homologues and glomangiomas. For this reason, as discussed previously, it should be called the "glomus" cell and not the "epithelioid" cell. Such cells are round or polygonal in shape. The cytoplasm is clear or pale staining but does not contain glycogen, fat or mucin. In the most carefully preserved Zenker-fixed material in the tumors of this series the cytoplasm takes a faint, homogeneous acid stain. There are no external or internal myofibrils. A few of the cells contain small granules, present after both Zenker and formalin fixation and in sections embedded in celloidin and paraffin. They stain deeply with basic dyes and are brought out clearly as black dots in the Cajal reduced silver preparations. The cytoplasm is brownish after fixation in chrome salts. In outline and in nuclear details these cells correspond to those adjacent to them. The appearance may represent an early degenerative change. The nuclei of all cells contain a large amount of chromatin which is arranged in a finely granular mat without the formation of a network. Mitoses are absent. These cells may be arranged in three ways. They may be closely packed together in the walls of vessels with single coarse collagen fibers intervening between adjacent cells. When arranged in this fashion there are groups of two or three nuclei at rare intervals which are separated by homogeneous cytoplasm without definite cell boundaries being demonstrable either with Mallory's aniline blue connective tissue stain or with Foot's modification of Hortega's silver carbonate method for reticulum. The cells also may be clumped in large masses without vascular lumens. In the third arrangement the cells are separated from one another by a homogeneous material which takes the same stains as collagen but somewhat lighter than the collagen fibers. In it the glomus cells are teased away from the larger masses. It is then that their outline is seen best and the delicate collagen fibers and nerve filaments are studied to particular advantage. Because of its intimate association with the glomus cells, it probably represents material produced by their degeneration.

This material is not found in the normal glomus though it seems rather characteristic of glomangiomas. These different arrangements of glomus cells result in great variability of general appearance in glomangiomas. Solid masses of cells alternate with areas in which cells are few, and homogeneous intercellular material is abundant. In still other portions, vascular lumens are so numerous and large that the tumors take on an appearance similar to that of the more common cavernous hemangioma. Yet even here close inspection shows that the cells making up the walls of the vessels are glomus cells and not the elongate smooth muscle cells of the hemangioma. In the cavernous portions of glomangiomas numerous nerve fibers are demonstrable with Mallory's phosphotungstic acid hematoxylin stain.

Among the glomus cells run large numbers of nerve trunks, mostly non-myelinated. At the periphery of the tumors the trunks are large and present a prominent Schwannian sheath. This is lost as the filaments ramify among the glomus cells, the nuclei becoming fewer the farther the nerve filament is followed from the periglomic trunks. The nerve fibers branch and terminate around glomus cells by the interposition of nerve endings. A nerve ending was illustrated by Mason and Weil.³² In the very fresh and carefully fixed material of Case 7 Cajal's reduced silver method shows that such nerve endings are more numerous. With the usual perversity of silver methods there are many areas in which no endings are seen but enough are impregnated to justify the statement that such is the method of termination. The nerve endings are composed of two or three minute filaments spread out over the glomus cells. They frequently have small nodosities or bulbous enlargements along their course, especially at their extremities. Sections stained with aniline dyes show the same rich plexus of nerves but give no indication of the method of junction of nerve and glomus cell. Masson^{33,34} stated that there is a syncytial relation between the two, a finding not confirmed in this material. Studies with Mallory's aniline blue connective tissue stain, Cajal's reduced silver method, and Masson's trichrome stain have given no support to the older views that the glomus cell is endocrine or angioblastic in nature.⁸

By choice of fields for study from different tumors and at different levels it is possible to follow the process of development of the various elements found in glomangiomas. The process of differ-

entiation is two-fold. Nearest a vascular lumen the cells are elongate; as one progresses centrifugally these become round in outline and lose their external and internal myofibrils. In areas without vascular lumens the elongate cells are at the center and the round ones at the periphery. The cytoplasm becomes homogeneous. The nuclei also become rounded and the chromatin arranged in a finely granular mat. At the same time the argyrophile reticulum investing the cells becomes coarser and is brought out intensely with aniline collagen stains. The second factor in differentiation is that of arrangement of the nerve fibers. Bundles of nerve fibers are gathered in large periglomic nerve trunks. These traverse the adjacent connective tissue and turn sharply to be incorporated in the tumors. Like the change in the smooth muscle cells, the nervous connections are established first at the periphery and development progresses toward the center.

The process of formation of the atypical Sucquet-Hoyer canals of glomangiomas consists of these two processes which go on simultaneously and which seem to be interdependent. In this way the two types of vessels described by Masson^{33,34} represent different stages of development of Sucquet-Hoyer canals. In the second type the process of differentiation has reached the lumen, while in the first type layers of elongate smooth muscle cells still remain.

The glomangiomas may be considered an overgrowth of a specific arteriovenous anastomosis and the neurones terminating in it. They thus represent proliferation of mesodermal and ectodermal elements. Masson⁴⁷ showed that pigmented nevi represent the proliferation of an entire end organ, in the dermis consisting of Meissner's corpuscles and chromatophores, and in the epidermis of Merkel-Ranvier corpuscles and chromatophores. A close analogy can be drawn, then, between glomangiomas and pigmented nevi. The comparison is exact only if one considers the chromatophores mesodermal. If subsequent investigations establish their ectodermal derivation then the nevi represent proliferation of tissue derived from only one germ layer.

The clinical syndrome produced by these tumors is as characteristic as their histological appearance. A summary of the clinical data of each case is given in Table I. The symptoms caused by each of the tumors were strikingly similar. Hence, the following case (Case 1) is described in detail as representative of the entire group.

REPORT OF CASE

Clinical History: A previously healthy barber, 48 years of age, was admitted to the hospital for removal of a painful tumor of the left upper arm. Twenty years before the patient sustained an injury to the outer side of the left upper arm at the deltoid insertion during a barroom scuffle. There was only slight immediate discomfort. However, for several weeks a spot could be noted at the site of the injury which, as time went on, changed color from red to greenish yellow to blue. The soreness about the area never entirely went away and after several months the region again became acutely painful. During the entire interval of 20 years a slight touch on this bluish nodule elicited localized sharp pain. If the patient did hard work with his left arm, particularly when the arm was held over his head, pain would be experienced about the anterior and posterior aspects of the shoulder, in the left pectoral region and on the left side of the neck. From the latter site it radiated toward the occiput. At such times the nodule became deeper blue and somewhat larger. He was able to sleep only if he placed his arm across his chest to prevent pressure on the nodule. For the two years preceding his admission he was unable to bathe his arm with any degree of energy. The patient found it necessary to protect his left upper arm when in a crowd because of the severe pain caused by inadvertent pressure. He worried a great deal about the condition. There was a loss of weight of 11 pounds in the 2 years. With the exception of the tumor, the general physical examination, including the neurological findings and roentgenograms, were normal. The tumor presented itself as a bluish area 3 mm. in diameter just above and anterior to the insertion of the left deltoid muscle. It showed little induration though the electrifying pain caused by palpitation made the examination unsatisfactory. The bluish area was removed with the patient anesthetized by nitrous oxide-oxygen. The operation was followed by immediate and permanent relief of pain.

The tumors are located usually upon the extremities, only 2 examples having been encountered elsewhere (Table II). One of these occurred over the acromion; the other, Case 6 of this series, was found on the lateral chest wall. Twenty-one of the 65 examples tabulated were located in the nail bed, 20 of them on fingers, 1 on a toe. This is a most unusual site for tumors, though Adair, Pack and Nicholson¹⁸ have pointed out several other types of tumors in that location. Most interesting of these is the melanoma, which shows great malignancy in this location, in contrast to the benignancy of glomangiomas.

The most characteristic clinical symptom of glomangiomas is pain. This may be spontaneous and intermittent or may arise only when the tumor is touched. The pain has a stabbing, burning character, described by one patient (Case 6) as that produced by a "red hot poker." It may often be elicited by the slightest pressure, as of clothing or of bedcovers. Patients develop curious habits associated with the protection of the tumors, such as carrying the hand in the

pocket to keep the trousers from touching a tumor on the thigh. A patient in this series (Case 6) for many years gave a history of awaking periodically from his sleep because of the pain. He would then get up and pace the floor, meanwhile holding his hand over the tumor so that his nightclothes did not touch it. The pain may also radiate over the extremity, shooting out from the tumor as a focal point. At times patients experience severe neuralgic pain but are entirely un-

TABLE II
Distribution of Glomangiomas

Lower Extremities	18
Thigh	9
Region of knee	2
Leg	3
Foot	2
Sole	1
Subungual	1
Location on extremity not given	2
Upper Extremities	44
Upper arm	4
Forearm	10
Hand	30
Thenar eminence	1
Palmar surface of finger	1
Dorsal surface of finger	1
Palmar fascia	1
Subungual	20
Location on hand not given	6
Acromion	1
Chest Wall	1
Location not given	1

aware of the tumor as an inciting point until on careful physical examination it is discovered (Case 7).

The tumors may be associated with sympathetic disturbances of vasomotor character, such as a Claude Bernard-Horner syndrome, as in Barré's²⁰ early case. He stated in the discussion of his original paper²⁰ that excision of the subungual tumor of the finger was followed in several days by the disappearance of the Claude Bernard-Horner syndrome.

Several cases have been described in which pain was produced when the patient went from a warm room to the cold outdoors. One patient (Case 7) suffered more severely when the finger bearing the tumor became cold than when pressure was made upon the tumor. Sometimes the pain has been relieved by immersion of the finger in

hot water.²⁸ One instance³⁷ showed a measurable difference in cutaneous temperature between the extremity bearing the glomus tumor and its fellow. Sympathetic disturbances originating in these tumors may have an unexpectedly wide distribution. The tumors may become more blue when painful than in the asymptomatic interval.

Let us now consider the mechanism of production of pain. Mason³³ believed it to be caused by pressure on adjacent pacinian corpuscles. This view has been generally accepted. Evidence can be gathered that the pain is associated with the dilatation of the glomic vessels. The increased blueness during paroxysms of pain is one point in favor of this view. At times, hard exercise with the extremity bearing the tumor (Case 1) causes pain, which might be explained by the increase in volume of blood passing through all vessels of the extremity. In consideration of the normal physiological reactions of the glomus it has been shown^{10,4} that when the temperature of the extremities is lowered, the glomic vessels are the first to dilate and may be the only ones to do so. Hence, cold would cause dilatation of the glomic tumor vessels. Those of the finger tips and distal phalanges are the first to respond,¹⁰ which fact might be correlated with the presence of some of the most painful of the glomus tumors in the nail beds. Normal glomic vessels dilate in response to trifling tactile or chemical stimulation of the cutaneous surface in the experimental animal.³ The dilatation of the vessels of glomus tumors, then, is associated with the paroxysms of pain. Moreover, it has been shown that there are many nerve fibers which enter the vessel walls and terminate about the glomus cells with nerve endings closely applied to their margins. The dilatation of the vessels would then set up impulses which would be carried to the large periglomic nerve trunks in the connective tissue surrounding the tumor. Further dissemination along these pathways might explain some of the more distant sympathetic disturbances which are relieved by the removal of glomangiomas. In this respect the glomangiomas behave functionally as overgrowths of neurovascular end organs, corresponding to their histological characteristics as such. That this radiating type of pain is only an exaggeration of the normal is shown by its close resemblance to that caused by prolonged immersion of the hand in ice water. The sensation of pain is not limited to the part of the hand in the water but extends to the elbow or even the shoulder in this

normal reaction to a strong stimulus just as it does in patients with a glomangioma, but in the latter case a much weaker stimulus will elicit the response.

The relation of a large number of glomangiomas to trauma is striking. In nearly half the cases some single severe injury is followed directly by the development of a glomus tumor. In Case 1 of this series a blow during a barroom scuffle was followed by a hematoma which gradually faded but never entirely disappeared, the bluish nodule slowly taking its place. Many similar instances are recorded as of a blow on the fingernail from the top of a desk³³ or injury to the knee in a bicycle accident.³² Numerous other types of trauma have been followed in the same manner by glomangiomas.^{40,31}

Since pain is a symptom which brings a tardy patient to the physician, it might be expected that treatment would be sought promptly. As a matter of fact, quite the contrary is the case. Of those examples in the literature in which the length of time from the onset of symptoms was stated (including the present series), the average duration was 14 years. In seeking an explanation we find that the symptoms increase slowly over many years. Perhaps the patients accustom themselves to the knife-like pains and pay little attention because of their familiarity. Furthermore, some patients do not recognize the tumor as a focal point for the pain, but believe that they suffer from "neuralgia" or similar malady. That such beliefs are not restricted to patients is shown by the fact that in one instance a periarterial sympathectomy was performed for the intractable pain with only temporary relief.³³ Some patients, also (Cases 6 and 7 of this series and that of Prodanoff³⁹) have been considered psychoneurotics and have been turned away without treatment until the tumor was recognized at a later date.

Local surgical excision produces complete and immediate relief from symptoms. The only instance in which there was any residual pain was that of Lortat-Jacob and Brosse,²⁹ reported shortly after operation. Local anesthesia is usually used in removal of glomangiomas. Interestingly enough a very large quantity may be required¹⁷ if local infiltration is done. Nerve block is very satisfactory and anesthesia by this method is as easily obtained as in other lesions of the same areas. Irradiation is without avail.¹⁷

Glomangiomas are entirely benign. No instance of recurrence has

been recorded. They are not invasive and are easily removed entire. This is of especial importance because extensive operations were often done when the nature of the lesion was not recognized, especially when a diagnosis of angiosarcoma was made. The immediate and permanent relief from intractable pain of many years duration makes these patients among the most grateful in the practice of surgery.

SUMMARY

The cutaneous glomus is an arteriovenous anastomosis in the stratum reticulare of the cutis, which is homologous with the glomus coccygeum and several less important vascular structures. These have in common a specialized glomus cell, which is a modified smooth muscle cell with abundant nervous connections. The cutaneous glomus has an important function as an arteriovenous shunt in maintaining the body temperature and perhaps the blood pressure.

From the cutaneous glomus, tumors arise which form a subgroup of the hemangioma. The term *glomangioma* is suggested for them to indicate their derivation and character.

Glomangiomas appear as small bluish nodules on the extremities or adjacent portions of the shoulder girdle. Very frequently they are located in the nail bed. Microscopically the tumors are composed of cells identical with those in the walls of the normal cutaneous glomus and its homologues. Nerve trunks are numerous in the connective tissue about the tumors and nerve filaments pass among the glomus cells in large numbers. Occasionally elongate smooth muscle cells are seen either in solid masses or adjacent to vascular lumens.

The glomangiomas represent the overgrowth of the entire arteriovenous anastomosis and in doing so their cells show a two-fold differentiation. The elongate smooth muscle cells lose all myofibrils, while the reticulum investing them becomes much coarser and stains intensely with collagen stains. Secondly, the periglomic nerves grow into the tumors and their terminal filaments end about the differentiating smooth muscle cells with the interposition of nerve endings. These two processes result in the formation of the glomus cells and are apparently interdependent.

The tumors are associated clinically with severe radiating pain of neuralgic type. In character and distribution this has many similarities to the response of the normal glomus to much greater stimuli of

the same character. Glomangiomas thus represent functionally as well as morphologically organoid overgrowths.

Glomangiomas do not become malignant. Local excision gives complete and permanent relief from symptoms.

NOTE: I wish to express my thanks to Dr. Monroe Schlesinger of the Beth Israel Hospital, Boston, Mass., for the material in Cases 2 and 3. I am also indebted to Drs. Sutton and Klinck of the Albany Hospital, Albany, N. Y., for the pathological material of Case 5. They will publish a paper on the clinical aspects of that case separately.

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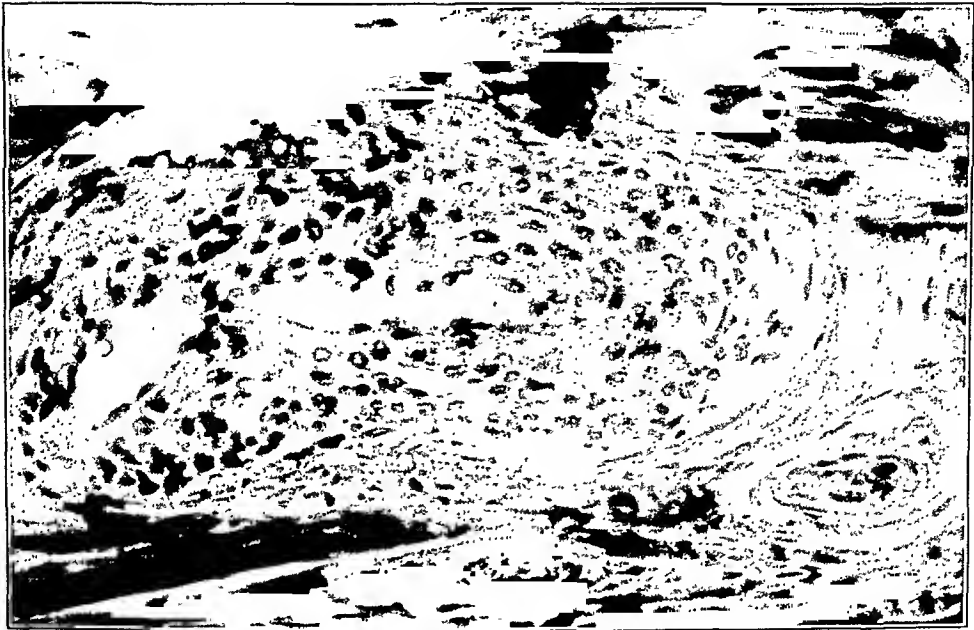
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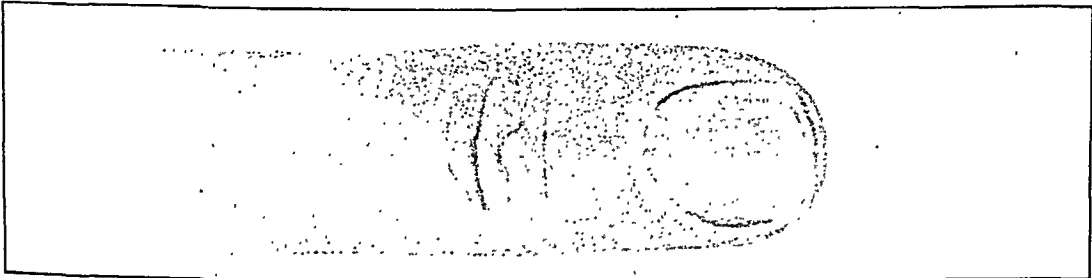
DESCRIPTION OF PLATES

PLATE 121

- FIG. 1. The normal cutaneous glomus. The specimen was obtained from the pad of the great toe of a man 36 years of age. The Sucquet-Hoyer canal has been cut longitudinally and is surrounded by a thick layer of glomus ("epithelioid") cells. At the top, to the left of the Sucquet-Hoyer canal, there is a periglomic nerve trunk. Dense connective tissue fibers surround the glomus. Mallory's phosphotungstic acid hematoxylin stain. $\times 215$.
- FIG. 2. Drawing of a subungual glomangioma (Case 7). There is no elevation or erosion of the nail. The tumor, while sharply demarcated from the surrounding tissues, is somewhat irregular in outline.
- FIG. 3. The general appearance of a glomangioma at low magnification (Case 6). There are many irregular vascular lumens surrounded by glomus cells. Adjacent vessels are separated from one another by homogeneous material. The margin of the tumor is very sharply defined. Mallory's phosphotungstic acid hematoxylin stain. $\times 160$.



1



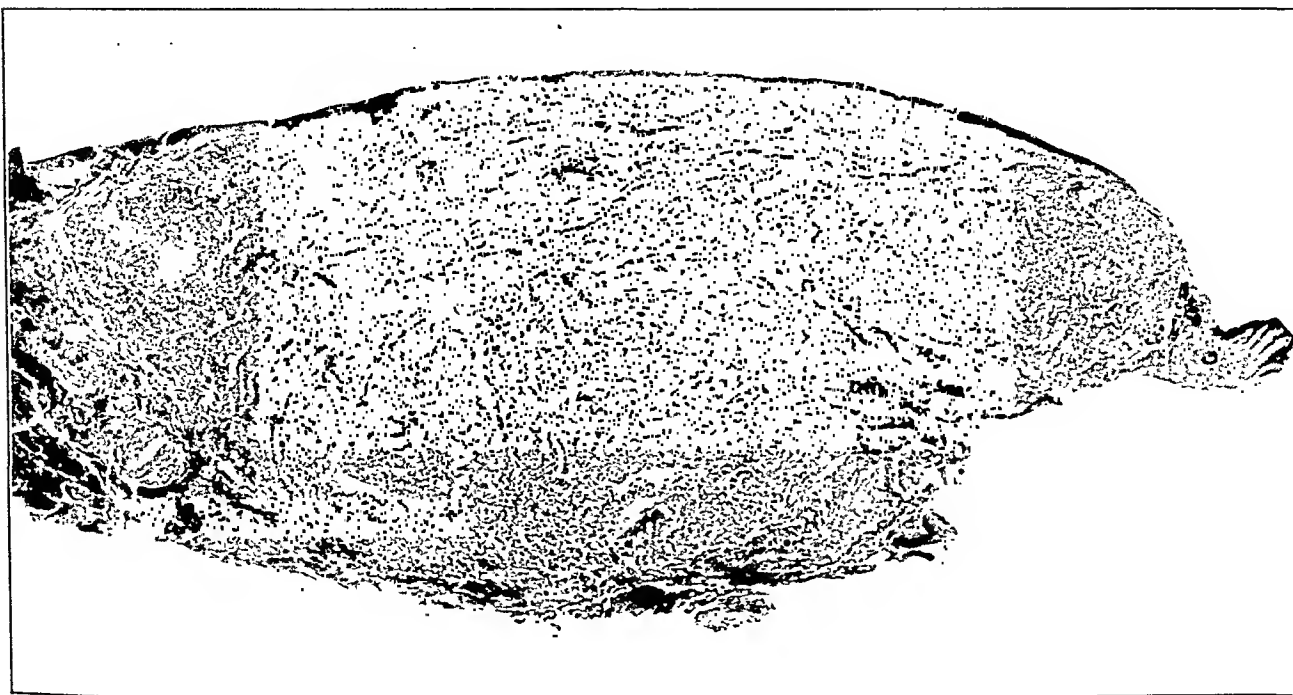
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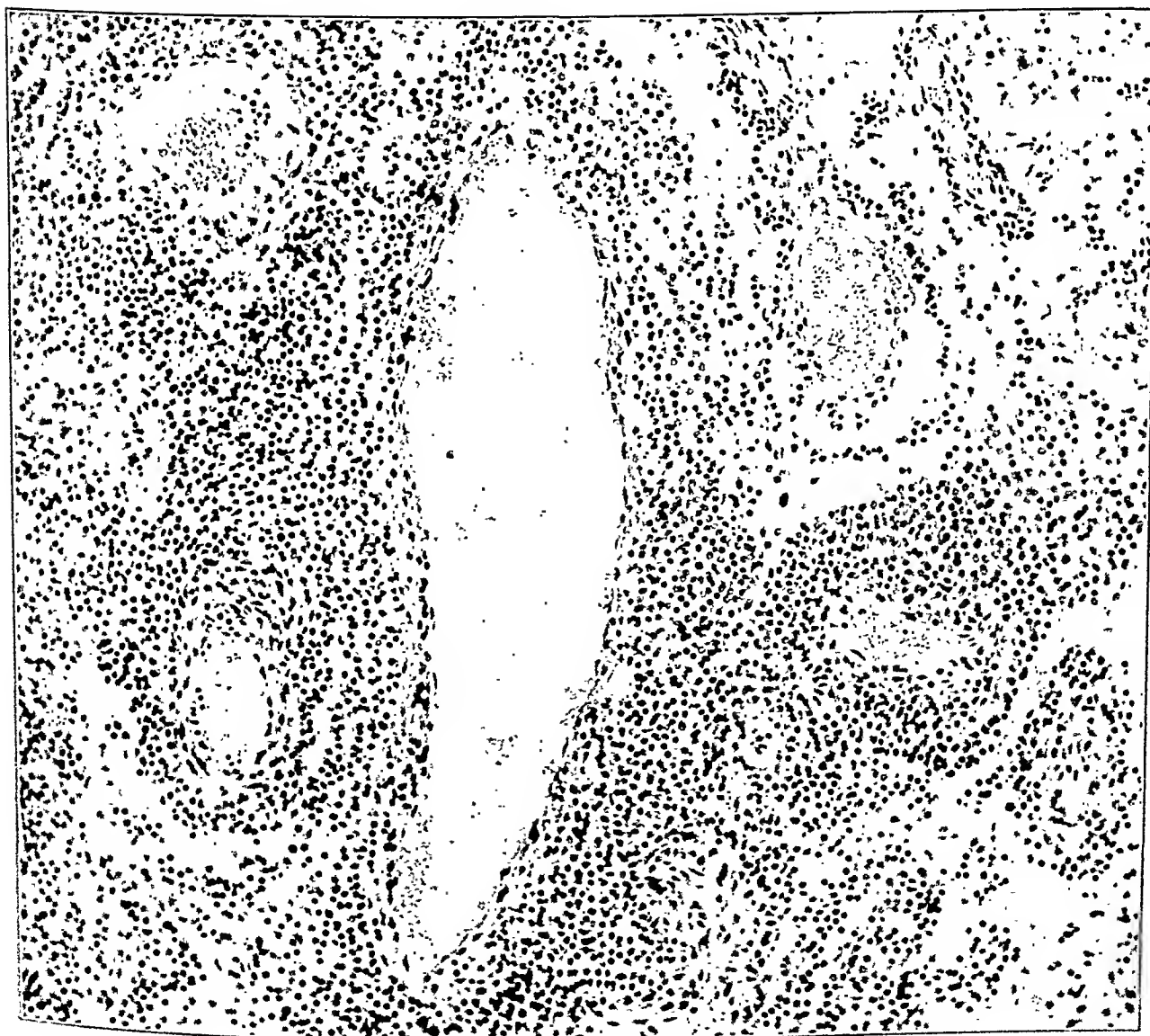
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PLATE 122

- FIG. 4. Section through an entire subungual glomangioma (Case 7). The uniform contour of the overlying nail bed is seen at the top. The sharp demarcation of the tumor from the subcutaneous tissues is also shown. Mallory's phosphotungstic acid hematoxylin stain. $\times 12$ (slightly reduced).
- FIG. 5. The vessels of a glomangioma (Case 5). The endothelium is surrounded by glomus cells. Some of these are separated by the homogeneous material and lie embedded in it. Hematoxylin and eosin stain. $\times 200$.



4

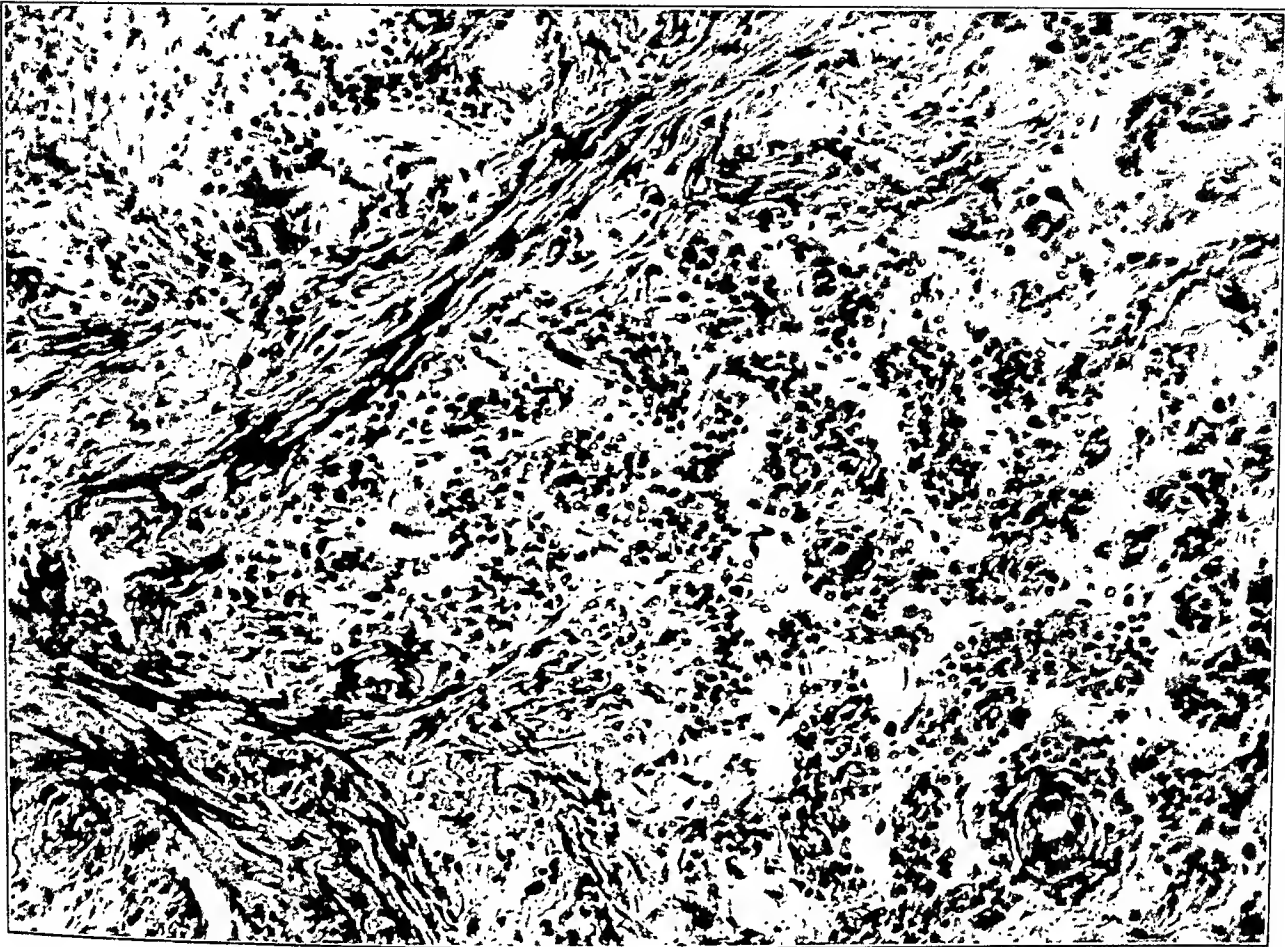


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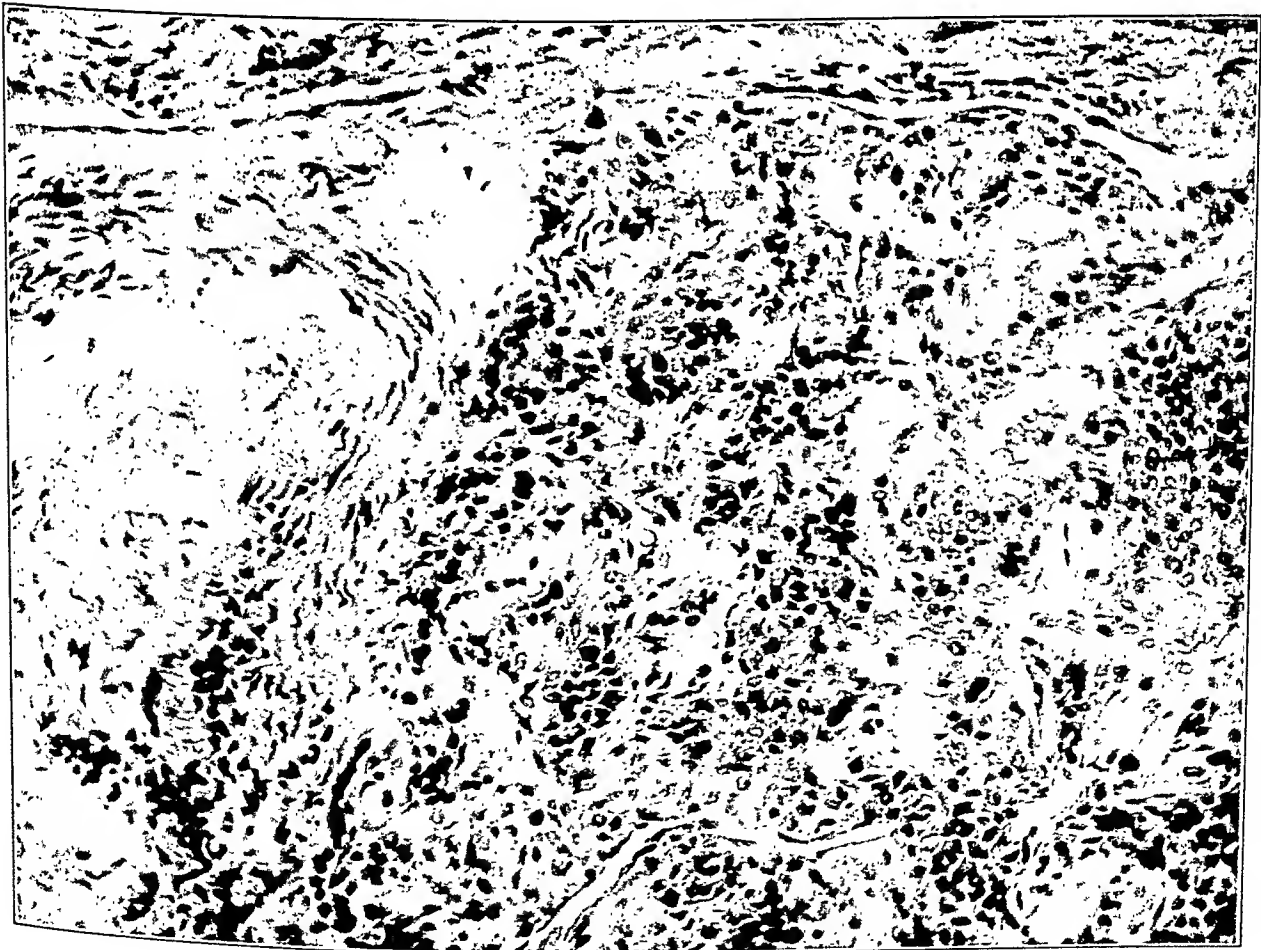
PLATE 123

FIG. 6. Large nerve trunks within a glomangioma (Case 5). Mallory's phosphotungstic acid hematoxylin stain. $\times 200$.

FIG. 7. The entrance of a nerve trunk to a glomangioma (Case 7). A large periglomic nerve trunk bends abruptly and becomes incorporated in the tumor. Hematoxylin and eosin stain. $\times 175$.



6

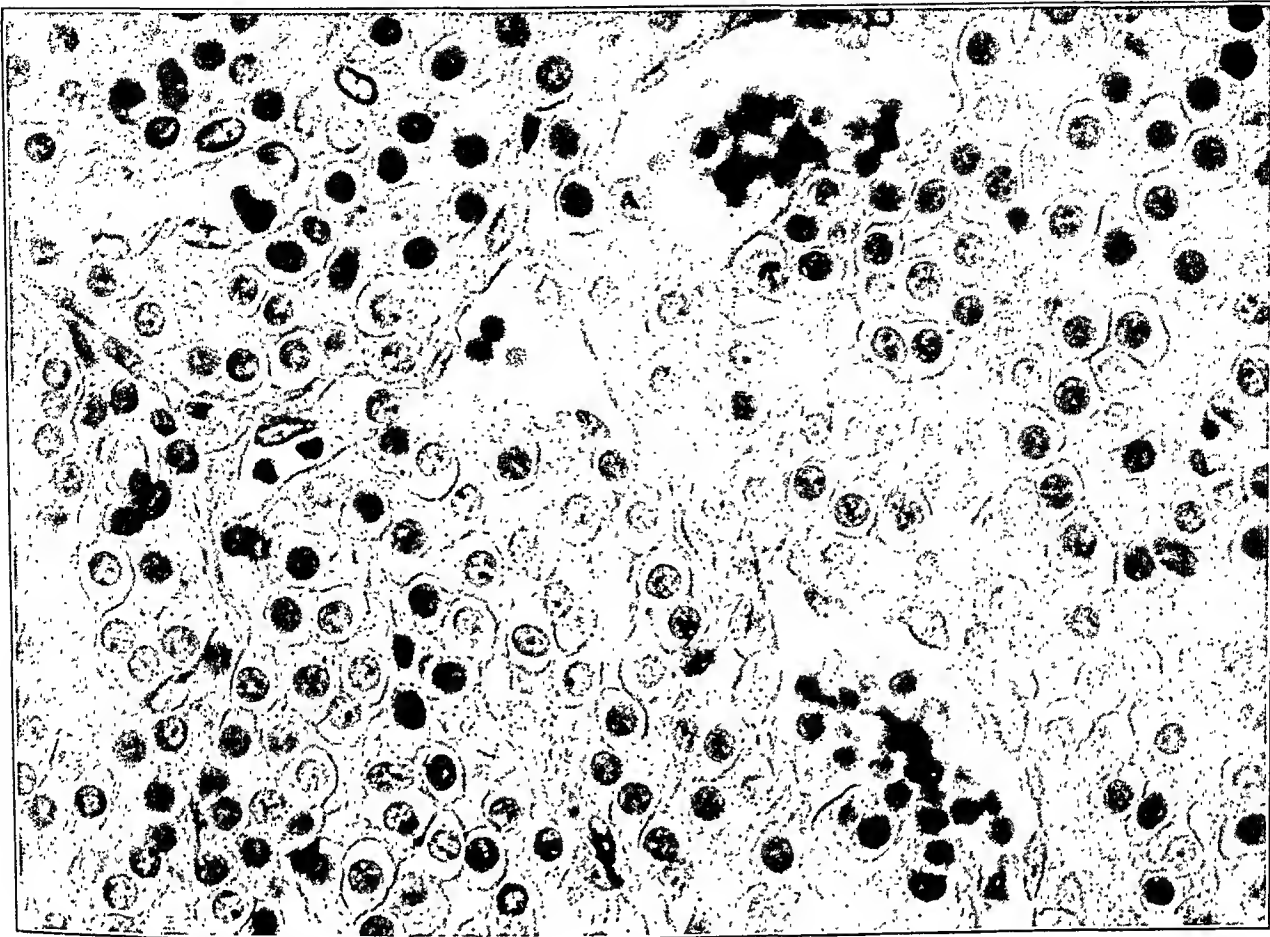


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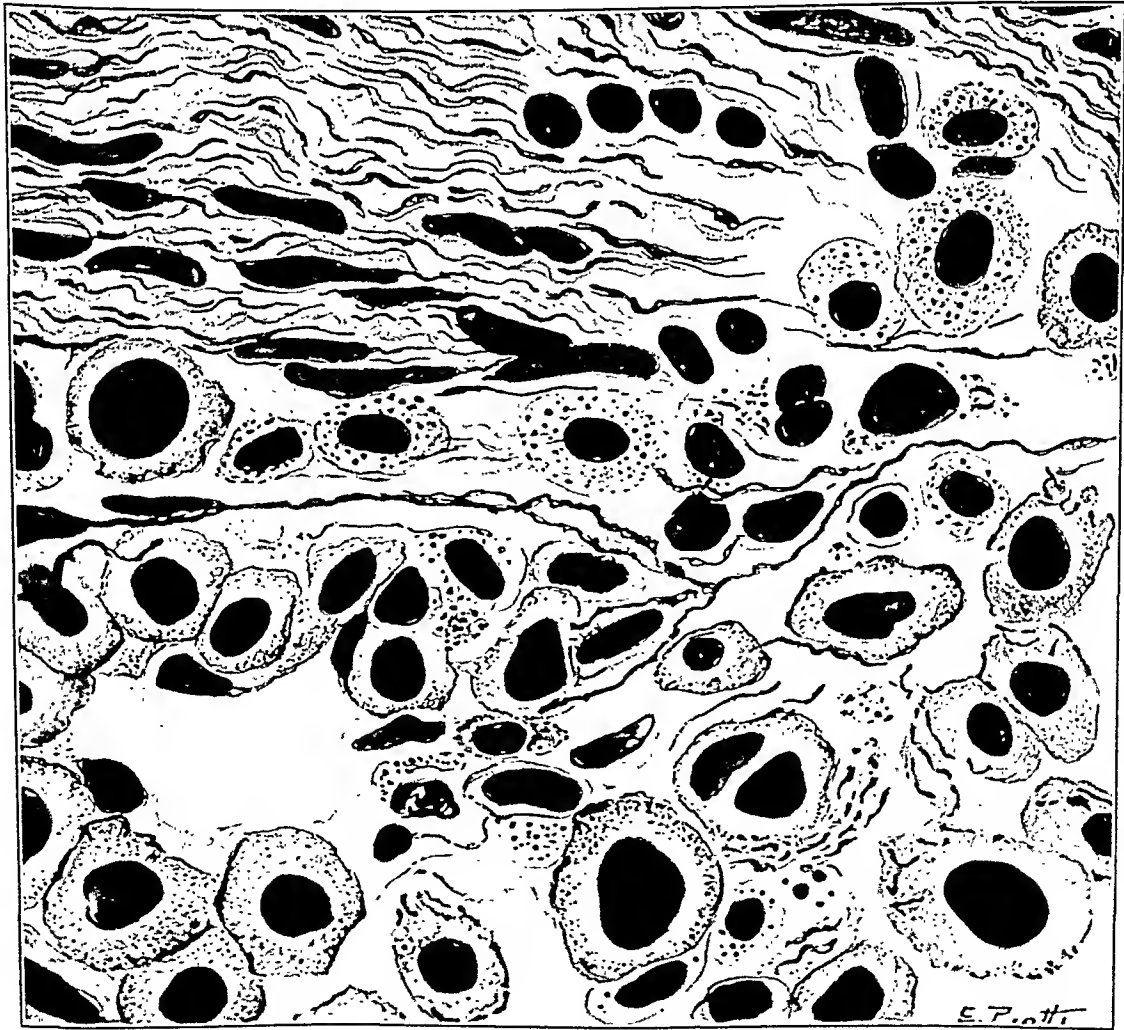
PLATE 124

FIG. 8. A mass of glomus cells with occasional vascular lumens (Case 6). The nuclei show uniformly arranged chromatin. The cytoplasm is homogeneous and surrounded by a prominent limiting membrane. Mallory's phosphotungstic acid hematoxylin stain. $\times 720$.

FIG. 9. Camera lucida drawing of a nerve trunk and glomus cells (Case 7). A large nerve trunk with Schwannian sheath is shown at the top of the illustration. Its fibers extend among the glomus cells. A few glomus cells show nerve endings. Several contain cytoplasmic granules stained by the silver. The vascular lumen is lined by a single layer of flattened endothelial cells. Cajal's reduced silver method. $\times 1200$.



8



9

CORNEAL REACTIONS OF NORMAL AND OF TUBERCULOUS GUINEA PIGS TO TUBERCULO-PROTEIN AND TUBERCULO-PHOSPHATIDE *

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Tissue reactions to the more purified components of the tubercle bacillus indicate that the protein fraction is responsible in a large measure for the acute exudative reaction, including much of the cellular response to the organism, and that the lipoids, particularly the phosphatide, stimulate the more chronic response and particularly the development of epithelioid cells and the lesions characteristic of tuberculosis.

Following intraperitoneal injections of adequate amounts of tuberculo-protein in normal rabbits, Doan, Sabin and Forkner¹ and Miller² observed an outpouring of leukocytes, clasmatoocytes, plasma cells and lymphocytes in the omentum. A similar cellular response was found in the meninges of normal and of tuberculous rabbits by Bickford.³ In some of the omentums studied by Miller plasma cells predominated. In general, they were noted by all of the above investigators as a prominent feature of the reactions. Epithelioid cells were never present in significant numbers. After intratracheal injections of tuberculo-protein Larson and Long⁴ observed an exudation of polymorphonuclear and mononuclear leukocytes into the pulmonary alveoli of both normal and tuberculous guinea pigs, the more marked reaction occurring in the latter group. Seibert⁵ noted a slight leukocytic response in the skin of normal guinea pigs following an injection of purified tuberculo-protein. In tuberculous animals and in those sensitized by tuberculo-protein the cellular reaction was marked even to very small amounts of the material; it consisted of many polymorphonuclear neutrophils, large numbers of eosinophiles and mononuclears. Another striking change, as Long⁶ had pointed out previously, was the marked swelling and subsequent degeneration of the connective tissue fibers. In the testes of tuberculous and tuberculo-protein sensitized guinea pigs Seibert found an interstitial edema and infiltration by numerous mononuclear exudate

* Received for publication June 26, 1935.

cells in response to the protein. In many instances there were polymorphonuclears and eosinophiles also. Some testes showed marked degeneration of the germinal cells. Similar changes had been noted by Long ⁷ after injecting Old Tuberculin into testes of tuberculous guinea pigs. In the later stage of the reaction these organs became atrophic and were infiltrated with large mononuclears. Old Tuberculin was without effect on testes of normal animals. The protein used by Seibert caused a slight leukocytic infiltration in the testes of some normal animals and none in others.

These results have shown that though relatively inert in normal tissues in small amounts, tuberculo-protein in sufficient quantity is capable of stimulating a cellular response somewhat similar to that to the same material in tuberculous animals, except in degree. In the allergic tissues is the added factor of tissue degeneration in many cases.

The biological testing of the various lipoids isolated from the tubercle bacillus has shown that the phosphatide fraction stimulates the most specific cellular reaction. Sabin ⁸ has given a comprehensive review of the literature concerning the activity of the tubercle bacillus lipoids in animal tissues and has summarized the extensive experiments performed by herself and her co-workers with these substances. It was found that repeated injections of the phosphatide into the peritoneal cavities of normal rabbits resulted in the production of masses of epithelioid cells, some of which were arranged to form typical tubercles. Giant cells and caseation were also observed. Injected into the subarachnoid space of normal and of tuberculous rabbits, phosphatide from bovine tubercle bacilli caused a slow production of epithelioid cells.³ On the other hand, Boissevain ⁹ found evidence that tuberculo-phosphatide, if highly purified, did not bring about development of epithelioid cells or tuberculous-like tissue. Such were present when a water-insoluble protein of the tubercle bacillus was used, however. Smithburn and Sabin ¹⁰ found no tuberculous-like tissue after injecting water-insoluble protein derived from tubercle bacilli. Intracutaneously, a rather persistent nodule without any likeness to a tuberculin reaction was produced by tuberculo-phosphatide at the site of injection in tuberculous animals.¹⁰

Since no parallel studies had been made of the action of small amounts of tuberculo-protein and tuberculo-phosphatide in normal and allergic animals, these were undertaken. Because of its access

for continuous observation grossly, and since its avascular structure offered a favorable site for tracing inflammatory cells, the cornea was chosen as the tissue in which to study the reactions to the above materials. Guinea pigs were used. The protein and phosphatide employed were prepared from a human strain of the tubercle bacillus.

MATERIALS

The protein was the purified TPT prepared by Dr. Florence B. Seibert from tubercle bacilli of the H37 strain by the ultrafiltration and trichloroacetic acid method.¹¹ The phosphatide, A3-a, was also prepared by Dr. Seibert according to the method of Anderson¹² from an alcohol and ether extract of strain 119 tubercle bacilli, which was in fact a strain of H37 from another laboratory. Additional filtrations through Seitz filters were employed for removing remaining bacillary debris. There were no acid-fast particles in the final product, which easily made a permanent fine emulsion in distilled water and in normal saline solution. It contained 0.229 per cent of nitrogen of undetermined nature.

METHOD

A group of male guinea pigs that had been infected for 6 to 8 weeks with H37 tubercle bacilli, and which gave strong tuberculin reactions, received intracorneal injections of approximately 0.01 mg. of tuberculo-protein TPT in 0.005 cc. of normal saline. A group of normal guinea pigs was similarly injected. Another group of tuberculous and one of normal guinea pigs received approximately 0.1 mg. of the tuberculo-phosphatide A3-a emulsified in 0.005 cc. of normal saline in the cornea. One animal from each group was killed by illuminating gas at 3, 7, 15 and 30 days after injection. At later dates the experiment was repeated twice, with constant results. In one repetition animals were sacrificed at 30 hours and 11 days in addition to the above periods. The injected eyes were removed, fixed in formol-Zenker's solution and prepared for sectioning by the celloidin method. Hematoxylin and eosin stains were used routinely; hematoxylin-eosin azure and a modified Weigert fibrin stain¹³ were used in certain cases.

In the subsequent descriptions the term mononuclear cell will include the various types of uninuclear cells of inflammation except

epithelioid and plasma cells. This is used because of the difficulty in accurately identifying many cells which were of intermediate or transitional types. The cells referred to as epithelioid are mononuclears with a large amount of pale, finely granular cytoplasm and a large vesicular nucleus, their shape being round to elongate, depending apparently on the degree of pressure by adjacent structures. These cells are like those seen in typical tuberculous lesions. The term fibrinoid is used to describe fibrin-like material in the anterior chamber of certain eyes which did not stain like fibrin by special methods for demonstrating this substance.

REACTIONS TO TUBERCULO-PROTEIN

Normal Animals: Grossly at 30 hours there was a slight central cloudiness in the corneas of normal guinea pigs. This diminished rapidly and disappeared by or shortly after the third day.

Microscopically the reaction at 30 hours consisted of numerous polymorphonuclear neutrophils between collagen bundles in a small central area of the cornea, while a few such cells were to be seen in the limbus and migrating toward the center. At 3 days small numbers of cells, a few of them mononuclears, remained, but at subsequent periods the tissue was essentially normal.

Tuberculous Animals: Grossly the cornea of the tuberculous guinea pig sacrificed 30 hours after injection was diffusely grayish white and semitranslucent. At 3 days new capillaries could be seen at the sclerocorneal junction, and the grayish white appearance was more intense, especially 1 to 2 mm. from the corneal margin. By 7 days many vessels were approaching the center, which was now white and almost opaque. Between the 9th and 15th days ulcerations with vascularized margins developed in the opaque area in seven of eight animals. The reaction in the periphery was diminishing. At 30 days all that remained was a poorly demarcated, barely visible, gray, central area traversed by a few small blood vessels.

Microscopically at 30 hours the epithelium was missing from the center of the cornea of the tuberculous guinea pig. This cornea was thickened three to four times normal because of a marked swelling of the collagen bundles and some interstitial edema; the latter was more pronounced in the limbus, where large numbers of polymorphonuclear neutrophils were present in the interstitial spaces. Among them, but more numerous about the vessels than elsewhere, were

many mononuclears of all types from small lymphocytes and other less differentiated small cells to large, round or irregularly shaped cells with large, oval to bilobed nuclei, some containing relatively small amounts of chromatin, others coarse, dark chromatin strands. A few well defined spaces adjacent to blood vessels, perhaps lymphatics since they contained no erythrocytes, were packed with these cells and a few polymorphonuclears. Fibrous connective tissue cells and endothelial cells were swollen. Large numbers of polymorphonuclears had migrated into the cornea proper for a short distance, but few had reached the center, where swelling of the collagenous tissue was the only striking feature. In the anterior chamber, enmeshed in a delicate fibrinoid network, were many polymorphonuclears and an occasional mononuclear. Numerous cells of similar types were in the iris and its angular space.

At 3 days (Fig. 1) masses of polymorphonuclears lay in the interstitial spaces well out in the cornea but were still sparse in the center. They had decreased markedly in the limbus, mononuclears being predominant and increased greatly in number, particularly about the vessels. In some instances they filled well defined perivascular spaces. There was a definite proliferation of new capillaries, in the walls of which endothelial cells in mitosis were found frequently. The lumens of these capillaries contained polymorphonuclears and mononuclears considerably in excess of the normal number, the ratio of the two types being approximately one to one. Examples of migration of these cells through vessel walls were present. The anterior chamber contained many more leukocytes of all types and a more coarse fibrinoid network than previously. There was little change in the iris and the angular spaces. Numerous leukocytes were clustered about the ciliary body.

At 7 days many newly formed capillaries were approaching the center. Throughout the recently vascularized areas mononuclears were numerous and definitely outnumbered polymorphonuclears, while in the non-vascularized center they were still scarce. They predominated over polymorphonuclears in vessels as well, and migration of both types of cells through endothelium was taking place. In the corneal center were large numbers of polymorphonuclears, most of which were degenerating, as indicated by swollen granular cytoplasm and pyknotic nuclei. Some had been phagocytosed by the few large mononuclears present and by other polymorphonuclears.

In one cornea cells of the latter type were confined chiefly to a well localized area, while in all others they were spread diffusely through the tissue. In the limbus, inflammatory cells had diminished and throughout the cornea swelling of collagenous tissue was receding. The inflammatory process was subsiding in the anterior chamber also. Plasma cells in moderate numbers appeared in the iris of one eye.

In the one cornea studied at 11 days the chief change was an extensive ulceration and sloughing of necrotic collagenous tissue and degenerating inflammatory cells in the center. In adjacent areas the cellular constituents were mostly mononuclear, some of which were large with pale cytoplasm and large vesicular nuclei; apparently these were approaching an epithelioid-like state. There was no evidence at this or at earlier periods of local mononuclear cell formation. The few mitotic figures present were of endothelial or of fibroblastic origin as far as could be determined. In the limbus were relatively few cells. Approximately one-third were plasma cells, the remainder mononuclears.

At 15 days typical epithelioid cells and large numbers of more undifferentiated large and small mononuclears, many of them small lymphocytes, were spread diffusely through the corneal center. Whether large mononuclears and epithelioid cells were transformations of the smaller cells could not be positively determined; yet there were many intermediate forms which may well have represented successive stages of development from small mononuclears to the above types. The collagenous tissue in the center was stained lightly and had lost its laminated appearance in places, thus indicating early degeneration. The limbus was little altered from the previous period.

At 30 days the corneas were in various stages of change and recovery. In one the collagenous tissue formed a homogeneous matrix in the center. Here were suspended numerous epithelioid cells, large and small mononuclears, an occasional plasma cell and fibroblasts (Fig. 3). Phagocytosed cell debris was present in some epithelioid cells and in large mononuclears. In adjacent less degenerated areas small mononuclears were predominant. Again, transitional forms toward the epithelioid type were seen frequently. The blood vessels in the center contained fairly numerous, large and small mononuclears and a few polymorphonuclears. As at all previous periods,

some were migrating through vessel walls. Other corneas of this period showed distorted and thinned collagen bundles indicating previous injury and subsequent atrophy. The inflammatory cells had practically disappeared from these.

REACTIONS TO TUBERCULO-PHOSPHATIDE

Normal Animals: Grossly in corneas of normal guinea pigs tuberculo-phosphatide brought about a mild, but persistent, reaction characterized by a gray cloudiness which was confined to the area infiltrated with the phosphatide emulsion. The reaction reached its height at 3 days, then diminished slowly, but was still visible at 30 days.

Microscopically at 30 hours the center of the cornea was infiltrated with a moderate number of polymorphonuclear neutrophils, while in the limbus and migrating from it toward the center were fewer polymorphonuclears and an occasional mononuclear. Most cells of the latter type were about vessels in the limbus. There was no interstitial edema or swelling of collagen bundles. At 3 days a few mononuclears were to be seen in the center, where, however, the reaction still consisted of polymorphonuclears predominantly. At 7 days many of the latter cells were degenerating, and some had been phagocytosed by mononuclears of both large and small types, which had increased in number over the previous period. A very few were migrating from the limbus. In one cornea, in which the reaction was especially marked, perivascular spaces were filled with mononuclears. Some were also in vessel lumens, and a few were passing through the endothelium. By 15 days mononuclears predominated in the center. There were transitional forms between these and epithelioid cells, a few of which were present. At 30 days the latter type of cell was predominant and lay between collagen bundles in the center. They were present in varying number in every cornea receiving the phosphatide, in some cases occupying an area of considerable size. There were no typical tubercles.

Tuberculous Animals: In corneas of tuberculous animals the tuberculo-phosphatide reaction was intense and simulated that to tuberculo-protein in many ways. The marked difference in gross and microscopically was that the phosphatide reaction was localized rather than diffuse. In both normal and tuberculous animals it was more persistent also.

In gross at 30 hours there was a marked, circumscribed, grayish white opacity in the center of the cornea, while the peripheral portions were less involved and blue-gray. The height of the reaction was reached between the 3rd and 7th days. The central opaque area assumed a dense yellowish white color and was even more sharply circumscribed than before, appearing as a spherical nodule set deeply in the corneal tissue. Numerous capillaries that had appeared at the corneal margin by the 3rd day had almost reached the center by the 7th. The peripheral reaction was subsiding at the latter period. Between the 10th and 15th days two of ten corneas developed shallow central ulcers, which persisted but a short time. The dense central inflamed area was small and the vessels less prominent. At 30 days there was only a slight, but localized, central haziness much like that in the corneas of the normal guinea pigs sacrificed at the same period, but more marked than any corresponding corneas receiving the protein.

Microscopically at 30 hours the striking features of the phosphatide reaction in tuberculous guinea pigs were the marked swelling of collagen bundles and the large number of polymorphonuclear leukocytes that filled the interstitial spaces throughout the center of the cornea. The swelling referred to was identical with that described in the tuberculo-protein reaction. The cell types were the same as well. In the limbus, mononuclears and polymorphonuclears were decidedly less numerous than in response to the protein. The same was true of the iris and its angular spaces, and there was almost no involvement of the anterior chamber.

At 3 days (Fig. 2) capillaries were proliferating extensively in the limbus and were pushing into the cornea for short distances. They contained increased numbers of mononuclears and polymorphonuclears in about equal proportions; a few of each type were passing through capillary walls. In the interstitial tissue of the limbus mononuclears showed a slight increase over the earlier period, especially about the blood vessels. In the center was a large, well localized area consisting of polymorphonuclears packed between distorted collagen fibers. Many of the cells showed signs of degeneration. The anterior chamber, iris and angular space were practically devoid of leukocytes, another indication of the definite localization of the reaction to the phosphatide in contrast with that to tuberculo-protein in tuberculous animals.

By 7 days new capillaries had reached the margin of the localized central cell mass, which now contained many mononuclears of all types. Phagocytosis of polymorphonuclears was much in evidence. In the adjacent vascularized tissue mononuclears were predominant; the same was true in the vessels. Between center and limbus as well as in the limbus itself were relatively few inflammatory cells.

In the cornea studied at 11 days the central zone was undergoing necrosis and ulceration. The adjacent vascularized tissue contained large numbers of mononuclears and, as described in the case of the corresponding animal receiving tuberculo-protein, many were approaching an epithelioid-like state. A few mature epithelioid cells were already present.

At 15 days the center was well vascularized, and epithelioid cells were numerous, if not predominant. There were still many mononuclears, especially in lateral portions of the cellular area. Some similar cells were migrating through vessel walls. The swelling of collagen bundles had practically subsided, and the limbus and other parts of the eye were essentially normal except for plasma cells in small numbers.

Thirty days after injection the corneas were identical with those of normal animals receiving phosphatide, except that a few receding vessels were still present in those of the tuberculous animals. There were epithelioid cells (Fig. 4) between collagen fibers in the center of each cornea, comprising a compact mass in some instances, but not arranged as a typical tubercle.

The marked tuberculin-like activity of tuberculo-phosphatide in corneas of tuberculous guinea pigs, in contrast with the mild reaction of normal guinea pigs to the same substance, suggested strongly that it contained tuberculo-protein. The difficulty, if not impossibility, of making an absolute separation of phospholipins and traces of protein is generally recognized, and Wells¹⁴ has called attention to the probability of the presence of some protein in most or all of the so-called lipid antigens of bacterial origin. Since foreign proteins mixed with lipoids often have greater antigenic power than when used alone,¹⁵ it was thought that perhaps the tuberculo-phosphatide may have enhanced the activity of any protein which it might contain. It was suggested that a study of the reactions to tuberculo-protein mixed with a phosphatide other than that from tubercle bacilli be made.

LECITHIN AND TUBERCULO-PROTEIN

Mixtures of 0.1 mg. of emulsified Merck's egg lecithin with 0.0001 mg. and with 0.00001 mg. of tuberculo-protein TPT were injected into the corneas of normal and of tuberculous guinea pigs. The same amounts of these substances were injected separately into corneas of other normal and tuberculous guinea pigs for control. Histological studies were made at 3, 7, 15 and 30 days after injection.

In the normal control animals tuberculo-protein, lecithin, and their mixtures brought about only a minimal reaction of polymorphonuclears and an occasional mononuclear without definite localization. Inflammatory cells were relatively most numerous at 3 days, the corneas being practically normal at subsequent periods. In tuberculous guinea pigs lecithin gave the same result as in normal animals.

There was no constant qualitative or quantitative difference between reactions to the lecithin-protein mixtures and to the equal amounts of tuberculo-protein alone in tuberculous animals. Intensity of the reactions varied with sensitivity of the animals. Some that were cachectic did not react at all; others that were highly sensitive responded almost as intensely and with the same types of cells, swelling of collagen bundles and vascularization of the cornea as did those receiving 0.01 mg. of tuberculo-protein. In most instances the reaction was diffuse, although in a few it was somewhat localized. The same was true of the lecithin-tuberculo-protein mixtures, a few animals injected with them not reacting at all and others strongly, but no more than to the small amounts of tuberculo-protein injected alone. Some reactions were diffuse with many leukocytes in the anterior chamber and limbus; others were confined to the cornea proper.

At 15 days some epithelioid cells and numerous mononuclears were seen in the center of the corneas of those animals responding most markedly, both to lecithin-protein mixtures and to 0.0001 mg. and to 0.00001 mg. of protein alone. The above types of cells were present in appreciable numbers only after vascularization of corneal centers had taken place. Mononuclears in the vessels were more numerous than normally, and some were migrating through the endothelium. As brought out several times previously, epithelioid cells seemed to be the result of transition from mononuclears after

the latter cells had reached the reaction site, chiefly through blood vessels.

In some corneas of the various groups of animals studied at 30 hours and 3 days after injection, a few small groups of bacteria were seen in or near the path of the needle. These organisms appeared morphologically like a micrococcus commonly found in air. They were without any significant effect on the reactions, since in those corneas in which bacteria were present the reactions were the same as those in other corneas of the same groups and periods in which none were found. None were seen later than 3 days after injection, indicating that, if present at first, they had been destroyed in every case after this period, and showing that they did not alter the reactions to the materials used for study.

DISCUSSION

In the above experiments swelling of collagen bundles in corneas of tuberculous guinea pigs receiving tuberculo-protein, also in those receiving tuberculo-phosphatide, was like that described by Seibert ⁵ in the cutaneous reaction of animals sensitive to the protein. The subsequent loss of structure and final partial atrophy of the collagen bundles in the protein reaction demonstrated the marked toxicity of tuberculo-protein for sensitized connective tissue. Cellular response to this material was, except for the presence of epithelioid cells, what one would expect in the reaction of any animal to an antigen with which it had been rendered allergic, namely, polymorphonuclear leukocytes at first, then large numbers of mononuclears. The appearance of epithelioid cells in appreciable numbers in later stages of the reaction was of significance, since the water-soluble protein fraction of tubercle bacilli had not been considered a stimulus for the development of these cells. Their presence in the reaction suggested that some of the epithelioid cells in lesions of tuberculosis may be due directly or indirectly to the action of tuberculo-protein on allergic tissues. Since there was no transformation to the epithelioid state of any of the few mononuclear cells responding to tuberculo-protein in corneas of normal guinea pigs, it would seem that the protein did not act on the mononuclear cells directly, but rather that some factor secondary to the degeneration of collagenous tissue or to degeneration of inflammatory cells brought about the change of

mononuclears to epithelioid cells. The real mechanism of the process is not understood.

The presence of epithelioid cells in later stages of reactions to tuberculo-phosphatide in corneas of both normal and tuberculous guinea pigs gave, without exception, further confirmation of the ability of this fraction of the tubercle bacillus to bring about the production of epithelioid cells, as Sabin and her associates have shown. Another important feature of the reactions was the early localization of responding cells in the center of the cornea, as opposed to the diffuse spreading of cells in tuberculo-protein reactions. The most logical explanation was that the phosphatide, as an emulsion, could not diffuse far from the site of injection because the globules of material could not pass through or between the collagen bundles; hence the central localization of reacting cells. The protein, as a solution, diffused from the cornea into the limbus, iris and anterior chamber, thus calling forth cells into all of these areas.

It seemed correct to assume the presence of tuberculo-protein in the phosphatide in view of its marked tuberculin-like activity in tuberculous animals, also because of its nitrogen content which, though small, might well have been due in part to protein. If this were true, then the localization of the allergic reaction to the phosphatide would indicate that the two substances were closely associated physically or chemically and that, as a result, most of the reaction due to the protein was confined to the area which the phosphatide penetrated at the time of injection. The failure of simple mixtures of lecithin and tuberculo-protein to give a localization of inflammatory cells also pointed to a close association of tuberculo-phosphatide with protein. Whether or not the phosphatide amplified the assumed protein activity by virtue of close association with it has not been determined. Because of the 0.229 per cent of nitrogen in the phosphatide, it is possible that 0.1 mg. injections of the latter contained as much as 0.0015 mg. of protein. This amount might well account for the difference in intensity between tuberculo-phosphatide reactions in normal guinea pigs and tuberculous ones.

In previous experiments ^{16, 17} it was shown that most of the mononuclears taking part in reactions to living tubercle bacilli injected into the corneas of guinea pigs and rabbits came into the cornea only after vascularization of it had occurred, and that these cells apparently migrated from the blood vessels. Some of them later differen-

tiated into epithelioid cells. These same observations have been made above in describing the reactions to the tuberculo-protein and phosphatide. It seemed that where there was but a slight irritation, as in the corneas of normal guinea pigs, sufficient numbers of mononuclears were able to migrate from the limbus without the aid of vascularization. Normally the limbus contains small numbers of lymphocytes and other mononuclears to supply some cells for mild injuries. In the presence of severe injuries, however, vascularization was apparently necessary to supply the needed mononuclear cells. Polymorphonuclears were able to migrate in large numbers from the limbus early in the reactions; mononuclears were not. In no case was there evidence of local proliferation of inflammatory cells, there being no mitotic figures except in endothelium and occasionally in cells which seemed surely to be fibroblasts. Neither was amitotic cell division observed. More positive indication of the vascular source of the mononuclears was their increased number in vessel lumens and the observation of some of them migrating through vessel walls. The fact that frequently they were most numerous about vessels was also in favor of their vascular entrance to the site of inflammation.

That epithelioid cells developed from mononuclears after these had reached the area of inflammation seemed to be demonstrated by the absence of epithelioid cells in the lumens of vessels; and the presence in the inflamed tissue of cells apparently in transition from mononuclear to epithelioid form further supported their mononuclear cell origin.

SUMMARY AND CONCLUSIONS

A study of the tissue reactions to purified tuberculo-protein and tuberculo-phosphatide in the corneas of normal and tuberculous guinea pigs was made over a period of 1 month. It was found that tuberculo-protein had a markedly toxic action on the connective tissue of corneas of tuberculous animals and led to inflammation and partial degeneration (tuberculin reaction). Furthermore, it seemed responsible, probably indirectly, for the production of epithelioid cells in the later stages of the allergic reactions. In the amounts used, the protein was practically inert in normal guinea pigs.

Tuberculo-phosphatide also caused an acute tuberculin-like reaction in tuberculous guinea pigs. Inasmuch as the preparation con-

tained a small amount of nitrogen, believed to be an impurity, and not part of the molecule, it was concluded that the reaction noted was probably a reaction to tuberculo-protein. The fact that the unknown substance was closely bound chemically seemed to explain the fact that the acute reaction to the tuberculo-protein alone was diffuse, while that to the tuberculo-phosphatide was localized. In both tuberculous and normal animals epithelioid cells were present at the later periods and persisted longer than did those in most of the tuberculo-protein reactions.

The findings confirmed previous work indicating that in tuberculosis of the cornea most mononuclears taking part in the reactions at the site of injection came from the blood stream, and that epithelioid cells arose from these mononuclears by a process of transition at the site of inflammation.

NOTE: The writer is indebted to Dr. Esmond R. Long for direction and other aid in the experiments presented and to Dr. Florence B. Seibert for supplying the tuberculo-protein and tuberculo-phosphatide.

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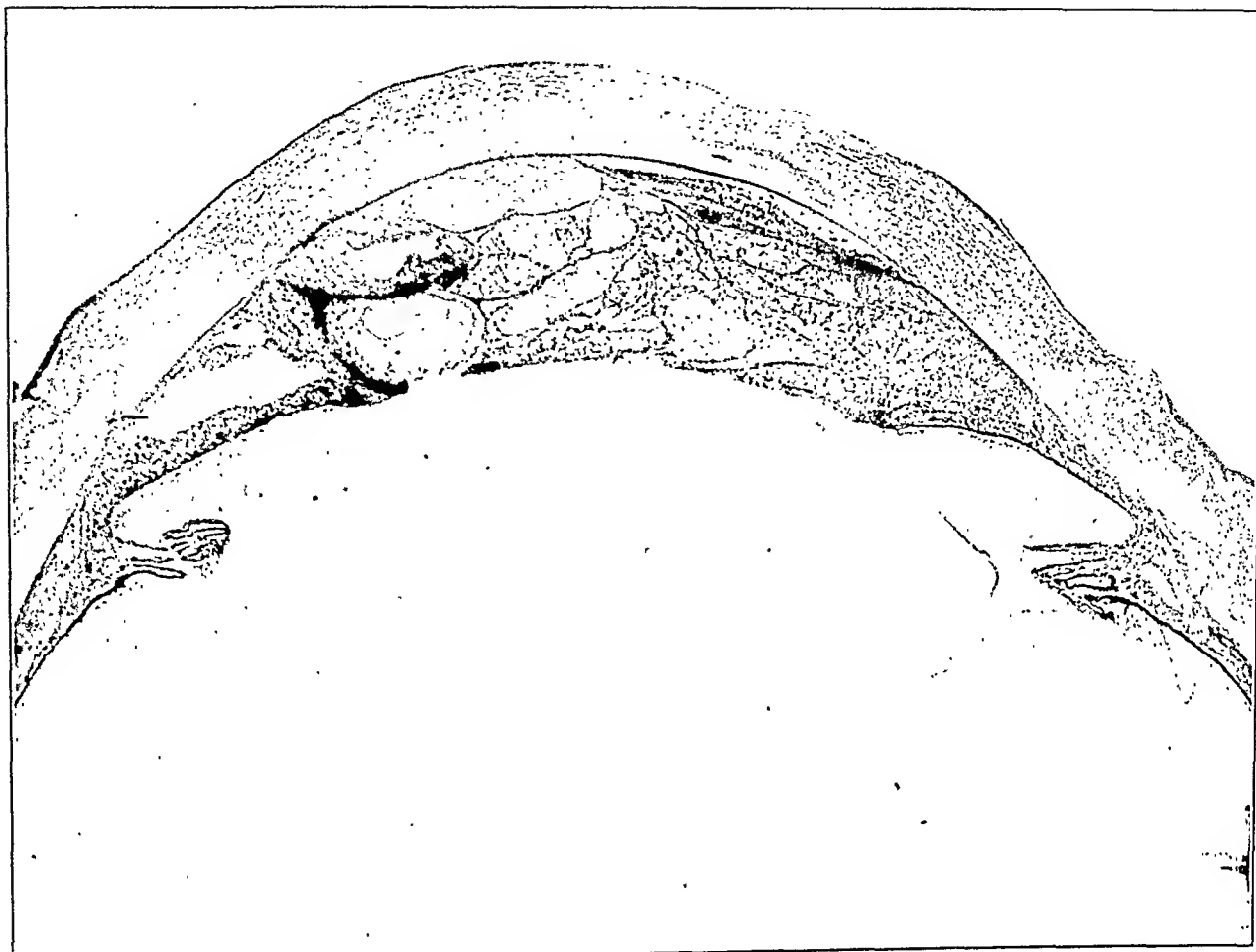
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DESCRIPTION OF PLATES

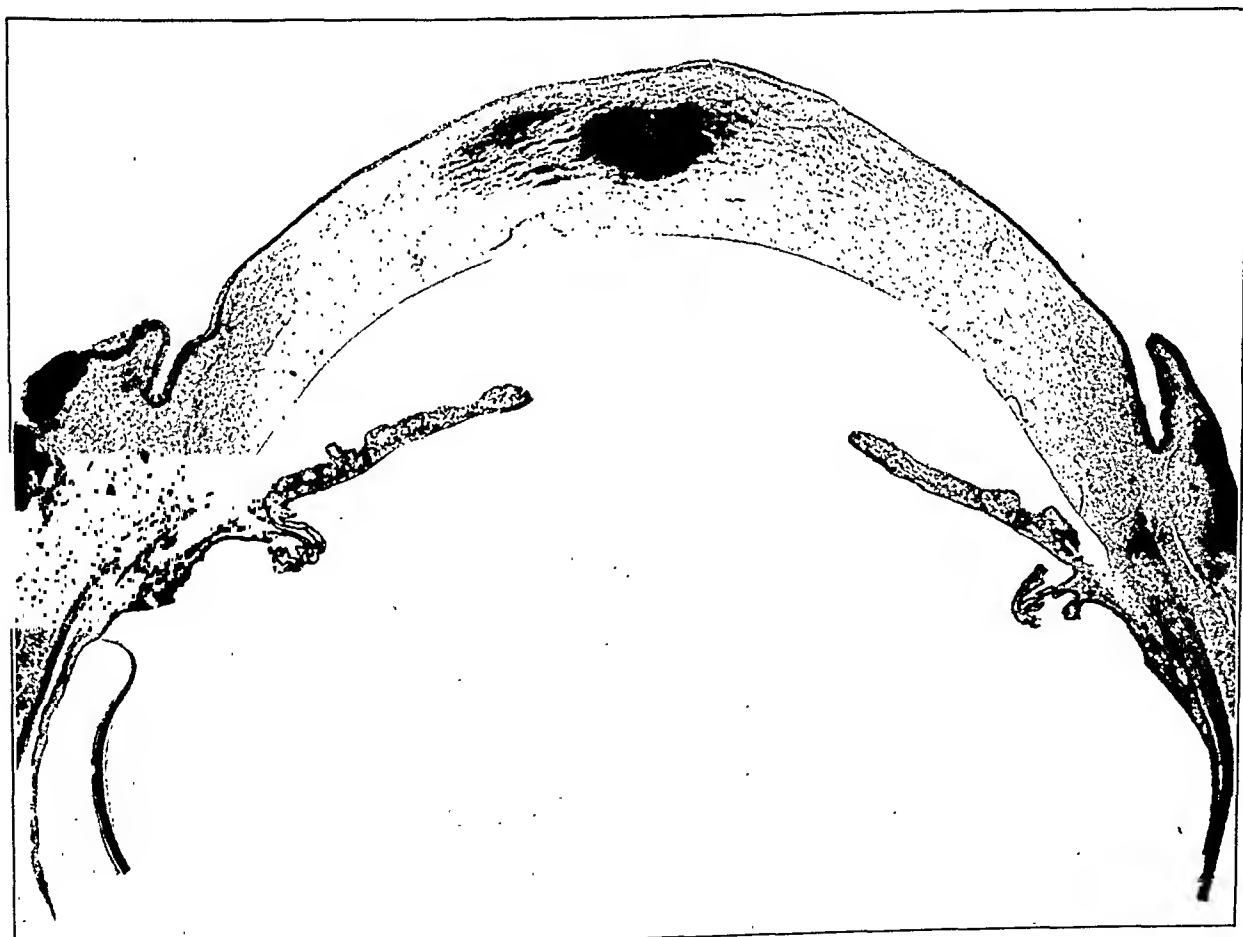
PLATE 125

FIG. 1. Cornea of tuberculous guinea pig 3 days after central injection of tuberculo-protein. Note marked density in and near limbus due to cellular infiltration, and relatively slight infiltration in center. Note also heavy exudate in anterior chamber. $\times 13.5$.

FIG. 2. Cornea of tuberculous guinea pig 3 days after central injection of tuberculo-phosphatide. Note dense cell "mass" in center and absence of exudate in anterior chamber. $\times 13.5$.



1



2

PLATE 126

- FIG. 3. Center of cornea of tuberculous guinea pig 30 days after central injection of tuberculo-protein. Note epithelioid and other less differentiated mononuclear cells in homogeneous matrix of degenerated collagenous tissue. $\times 910$.
- FIG. 4. Center of cornea of tuberculous guinea pig 30 days after central injection of tuberculo-phosphatide. Note large number of epithelioid cells and interspersed less differentiated mononuclears, some possibly in transition toward epithelioid type. $\times 910$.



3



4

THE SIGNIFICANCE OF THE CELLULAR VARIATIONS OCCURRING IN NORMAL SYNOVIAL FLUID *

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In order to establish the cytological characteristics of normal human synovial fluid studies have been made on human synovial fluid obtained immediately after death.¹ Such studies reveal considerable variation in the total number of nucleated cells as well as percentage differences of any one type of cell. Therefore, it is apparent that one cannot properly interpret these data until one has determined what cytological variations can occur and still be within the limits of normal. Because cellular variations of a lesser degree had been observed previously in bovine synovial fluid ² further studies of bovine fluid were undertaken in order to determine if possible not only what magnitude of cellular variation can exist without being considered abnormal, but also what factors are responsible for the observed cellular variations. In order to determine further the influence of certain factors on synovial fluid cytology some of the observations were extended to dogs because greater variations of the suspected factor were possible. Certain observed discrepancies between the present and previous bovine fluid studies ² were noted and have been recorded.

METHODS

Cell counts were recorded on 37 specimens of synovial fluid from young beef cattle. Of these, 25 specimens were obtained from the astragalotibial joint and 12 specimens were taken from the carpo-metacarpal joint. The method employed in the present study differed only slightly from that previously described.² It was found

* Read by Dr. Granville A. Bennett before the American Society for Experimental Pathology, April 11, 1934.

This is publication Number 17 of the Robert W. Lovett Memorial for the Study of Crippling Disease, Harvard Medical School, Boston, Mass.

The expenses of this investigation were aided in part by a grant from the Rockefeller Foundation.

Received for publication July 22, 1935.

that better differential stains were obtained if the concentration of vital dyes was reduced. Therefore, 12 drops of a saturated solution of neutral red in absolute alcohol and 4 drops of a saturated solution of Janus green B in absolute alcohol were added to 10 cc. of absolute alcohol. Clean glass slides were flooded with this diluted stain, drained and allowed to dry quickly.

In order to compare the phagocytic activity of each cell observed with its reaction to the vital dyes differential cell counts were made on two portions of each fluid, one of which contained a few drops of a graphite suspension. The same concentration of vital dyes was used in each type of preparation. The cell counts were then made simultaneously by two observers.

RESULTS

The results of the present series of cell counts are presented in Tables I and II. A comparison of the average percentage of the various types of cells listed in these tables with those previously published² reveals that there is a much higher percentage of non-phagocytic cells than we formerly reported. This group of non-phagocytic cells is comprised chiefly of cells having the characteristics of lymphocytes.^{3, 4} It will be noted that 20 to 23 per cent of all nucleated cells in the carpometacarpal joint fluid and 40 per cent in the astragalotibial joint fluid are lymphocytes, whereas we formerly reported that 90 to 95 per cent of all nucleated cells in synovial fluid from these two joints were phagocytic. The averages of the present tabulation indicate that only 74 to 78 per cent * of the nucleated cells in the synovial fluid from the carpometacarpal joint and 57 per cent of the nucleated cells in the astragalotibial joint fluid are phagocytic cells. These tables also show a wide range of variation in the numbers of phagocytic and non-phagocytic cells in different specimens of synovial fluid from the same source. No significant difference was noted between the average number of polymorphonuclear leukocytes and synovial cells in the two studies. A somewhat higher average of nucleated cells per cubic millimeter of astragalotibial joint fluid was obtained in the present series of counts. The former series of 63 specimens showed an average of 112 nucleated cells per cubic millimeter, whereas the average number of nucleated cells per cubic millimeter

* 74 per cent was the average for the fluids without graphite and 78 per cent represents the average for the same fluids when graphite was present.

of fluid in the present series is found to be 181. This difference is due chiefly to the fact that 4 specimens of fluid (Nos. 5, 13, 16 and 17) in the present series were unusually rich in cells.

DISCUSSION

Re-examination of the cells in the synovial fluid of cattle by means of supravital staining has demonstrated an error in our previously reported observations² in which 90 to 95 per cent of the nucleated cells were classified as phagocytes. The existing discrepancy resulted from a failure to distinguish between lymphocytes and small monocytes and from the belief that certain lymphocytes were shrunken degenerating cells. This source of error has been largely removed by reducing the concentration of vital dyes and by observing the function of each cell with regard to its ability to ingest particulate matter. In the previous study graphite was added to several synovial fluids for the purpose of making illustrations of the various types of cells contained therein. Had differential cell counts been made on these preparations we would not have made the mistake of classifying certain lymphocytes as monocytes.

The present series of differential counts was checked in each instance by preparations to which particulate matter had been added. Although the addition of a graphite suspension materially aided in the early stages of this study in the differentiation of phagocytic from non-phagocytic cells, it interfered greatly with the further classification of the phagocytic cells. This fact is clearly demonstrated by a comparison of the percentages of unclassified phagocytes counted in preparations without the addition of graphite, with the percentages of such cells obtained in counts made after such particulate matter had been added.

At the present time we know of no real value in subdividing the large mononuclear phagocytes of synovial fluid. However, an attempt has been made to classify them according to the criteria given for their differentiation⁵ in order to record the relative percentages of clasmotocytes and monocytes for future comparison with counts made on normal and pathological human synovial fluids.

In counting a specimen of synovial fluid one frequently encounters dead or degenerating cells. These are distinguished by their failure to react to vital dye, by their hyaline nuclei which are sometimes stained pale green, and by their shrunken or excessively vacuolated

cytoplasm. Such cells may resemble cells of the phagocytic series or they may resemble lymphocytes. Frequently, however, no clue to their identity is evident. All such cells have been excluded from these differential counts, although the number seen in counting 11 specimens of astragalotibial joint fluid and 7 specimens of carpometacarpal joint fluid has been recorded. From 2 to 24 such cells were seen in counting 100 viable cells.

Examination of Tables I and II reveals considerable variation in the average cell percentages in carpometacarpal and astragalotibial joint fluids. Seventy-four to 78 per cent of all cells present in the carpometacarpal joint are phagocytic, whereas in the astragalotibial joint fluid only 57 per cent are phagocytic. One notes further that there is less variation in the number of phagocytic and non-phagocytic cells present in carpometacarpal joint fluid. Is there any obvious explanation for the observed cellular differences in these two joint fluids? In a previous study⁶ it was noted that a degenerative type of articular cartilage defect is present in the carpometacarpal joints of all cattle over 2 years of age whereas, in so far as we could determine, no such articular cartilage lesion is present in the astragalotibial joints. Because of the presence of this cartilage defect the carpometacarpal joint fluids contain more débris and unidentified solid matter than the astragalotibial joint fluids. Such débris and particulate matter is removed from the joint chiefly by means of active phagocytic cells. Thus, it must be assumed that the increased mononuclear phagocytic cell content of carpometacarpal joint fluid represents the response necessary for the removal of the wear and tear products resulting from repeated trauma to this joint.

The variations in cell percentages observed in one astragalotibial joint fluid as compared with another were very great (Table II). The phagocytic cells varied from 36 to 92 per cent, whereas non-phagocytic cells (lymphocytes) varied from 8 to 64 per cent. Can variations of this degree be considered within the limits of normal? To answer this question it was necessary to consider all possible factors that might have been responsible for the variations noted. The following were considered and investigated:

- (1) Variations resulting from failure to withdraw the fluid at the time the animal was sacrificed.

- (2) Variations resulting from failure to examine the fluid immediately after its withdrawal.

TABLE I
Cytology of Synovial Fluid from Carpometacarpal Joints

Animal No.	Nucle- ated cells	Erythro- cytes	(A) Differential cell counts before the addition of graphite										(B) Differential cell counts after the addition of graphite																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																
			Phagocytic cells					Non-phagocytic cells					Totals					Phagocytic cells					Non-phagocytic cells					Totals																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																	
			Polymorphonuclear leukocytes	Monocytes	Clasmatocytes	Unclassified	nuclear phagocytes	Lymphocytes	Synovial cells	Unclassified	Phagocytic cells	Non-phagocytic cells	Dead cells seen in counting 100 nucle- ated cells	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent

(3) Whether or not the cytology of synovial fluid is altered by variations in the blood cytology.

Postmortem increases in the total number of synovial fluid cells have been reported by Key.⁷ He ascribes this increased cellular content as being due chiefly to a migration of polymorphonuclear leukocytes into the joint. The fluid cytology herein reported was done on fluids drawn 15 to 30 minutes after death because it had been established previously² that this lapse of time in no way influenced the total cell count or the individual cell percentages. Polymorphonuclear leukocytes were rarely observed.

In order to determine what changes in synovial fluid occur post-mortem, specimens of synovial fluid from the knee joints of four normal dogs were examined at varying intervals after death. Six fluids were obtained within the first half hour, 3 at the end of 1, 2½ and 3 hours respectively, and 4 at the end of 18 hours. In four instances sufficient fluid was obtained at a later aspiration (1 at the end of 2½ hours and 3 at the end of 18 hours) to repeat the total cell and differential counts. In 3 of these later fluids a marked increase in the total number of cells occurred without any appreciable alteration in individual cell percentages. Thus we are unable to ascribe percentage variations of any one type of cell as being due to post-mortem changes or intra-articular migration.

In order to determine the magnitude of variations in the differential cell counts which might result because of failure to examine the fluid immediately after its withdrawal a number of fluids were re-examined 2½ to 3 hours after the first count was made. Invariably such counts revealed a marked increase in the number of dead or degenerating cells. Considerable clumping of cells occurred when the fluids were kept in the warm box for such long periods of time. Aggregates of 5 to 50 cells were frequently observed. While the presence of such clumps did not alter the relative percentages of phagocytic and non-phagocytic cells in the specimens re-examined it was at times difficult or impossible to recognize the distinguishing characteristics of the phagocytic cells.

In the course of this study we observed in three instances cytological synovial fluid changes which suggested that the synovial fluid cytology might be a reflection of the blood cytology. For instance, in 2 pathological bovine synovial fluids an eosinophilic polymorphonuclear leukocytosis of 12 and 50 per cent respectively was noted.

TABLE II

Cytology of Synovial Fluid from Astragalotibial Joints

Animal No.	Nucleated cells	Erythrocytes	(A) Differential cell counts before the addition of graphite										(B) Differential cell counts after the addition of graphite									
			Phagocytic cells					Non-phagocytic cells					Phagocytic cells					Non-phagocytic cells				
			Poly-morphonuclear leukocytes	Monocytes	Clasmatoocytes	Unclasi-fied mono-nuclear phagocytes	Lymphocytes	Synovial cells	Unclasi-fied cells	Phagocytic cells	Non-phagocytic cells	Totals	Poly-morphonuclear leukocytes	Monocytes	Clasmatoocytes	Unclasi-fied mono-nuclear phagocytes	Lymphocytes	Synovial cells	Unclasi-fied cells	Phagocytic cells	Non-phagocytic cells	Totals
	per mm.	per mm.	percent	percent	percent	percent	percent	percent	percent	percent	percent	percent	percent	percent	percent	percent	percent	percent	percent	percent	percent	percent
1	135	225	6	32	16	4	42	0	58	42	42	..	2	18	18	18	18	40	2	56	44	..
2	140	25	3	33	2	0	62	0	38	62	62	..	0	18	8	8	64	64	2	34	66	..
3	85	10	0	30	30	2	34	4	62	38	38	..	0	20	16	28	32	32	4	64	36	..
4	125	40	0	38	24	8	22	6	70	30	30	..	0	12	8	24	52	52	4	44	56	..
5	395	60	0	18	16	2	62	2	36	64	64	..	0	16	14	12	58	58	0	42	58	..
6	170	50	4	29	23	8	34	0	64	36	36	..	0	10	13	32	40	40	2	55	45	..
7	105	105	0	12	28	0	60	0	40	60	60	..	0	20	13	23	39	39	5	56	44	..
8	105	12	0	24	24	0	50	2	48	52	52	..	0	4	16	30	44	44	4	50	50	..
9	140	5	0	32	36	0	28	2	68	32	32	..	0	24	18	22	36	36	0	64	36	..
10	0	42	12	0	44	2	54	46	46	..	0	16	16	22	46	46	0	54	46	..
11	85	935	6	44	12	0	34	2	62	38	38	..	0	16	6	20	58	58	0	42	58	..
12	230	10	8	28	22	16	24	0	74	26	26	14	4	22	28	18	24	24	2	72	28	..
13	370	640	7	54	28	3	5	1	92	8	8	7	8	30	24	30	8	8	0	92	8	..
14	115	50	2	38	14	4	42	0	58	42	42	8	6	30	16	24	24	24	0	76	24	..
15	175	65	2	30	10	14	36	2	56	44	44	6	2	26	10	16	46	46	0	54	46	..
16	575	45	2	46	6	8	38	0	62	38	38	4	2	26	6	26	36	36	0	60	40	..
17	315	5	0	50	12	2	36	0	64	36	36	2	0	28	4	38	28	28	0	70	30	..
18	160	45	0	36	8	0	50	4	46	54	54	8	0	28	10	16	42	42	0	54	46	..
19	85	5	2	32	6	0	58	0	40	60	60	28	0	14	6	18	54	54	2	38	62	..
20	55	90	2	52	4	4	36	2	62	38	38	14	0	16	4	35	41	2	2	55	45	..
21	260	45	4	40	0	0	56	0	44	56	56	26	0	10	0	34	54	0	2	44	56	..
22	125	50	0	60	2	0	40	0	62	38	38	14	0	18	6	24	48	0	4	48	52	..
23	60	740	0	48	12	5	34	0	66	34	34	6	0	20	10	36	30	30	4	66	34	..
24	120	95	6	28	16	8	40	0	58	42	42	16	2	12	4	48	32	32	0	66	34	..
25	130	35	*1	34	13	6	42	1	54	46	46	2	0	34	8	26	30	30	2	68	32	..
Max....	575	935	8	54	36	16	62	6	92	64	64	28	8	34	28	48	64	64	5	92	66	..
Min....	55	5	0	12	0	0	5	0	36	8	8	2	0	4	0	8	8	8	0	34	8	..
Aver. ...	181.8	141.1	2.2	36.4	15	3.9	40.1	1.2	57.5	42.5	42.5	11.1	1	19.5	11.3	25.1	40.2	1.5	1.3	57	43	10.2

* Eosinophile.

Because only one eosinophilic polymorphonuclear leukocyte had ever been observed in over 100 bovine synovial fluid examinations it was only natural to wonder if the above findings were indicative of an eosinophilia in these two animals. In addition, examination of the synovial fluid from a patient with myelogenous leukemia revealed that many of the synovial fluid cells were of the myelocytic series. If the synovial fluid does reflect the cytology of the blood under such conditions then there would be every reason to believe that diurnal variations in the synovial fluid cytology similar to those in blood cytology might occur.

In order to prove or disprove this theory the following observations were made:

(1) Simultaneous blood and astragalotibial synovial fluid examinations were done on eight additional cattle. These results are shown in Table III. One notes that variations of the total number of circulating leukocytes (4,200 to 10,800) or variations in number of any one type of cell can occur without any reflection of such variations in the synovial fluid. For instance, the polymorphonuclear leukocytes of the blood in one animal averaged 66 per cent, yet the synovial fluid contained none. In one other animal these cells averaged 2 per cent in the synovial fluid, even though the blood contained only 10 per cent. The same absence of relationship was demonstrable in the case of lymphocytes and eosinophilic polymorphonuclear leukocytes. The blood of each animal showed an eosinophilia varying from 2 to 12 per cent, yet no eosinophilic polymorphonuclear leukocytes were demonstrable in the synovial fluid.

(2) Similar studies made on simultaneously obtained canine blood and synovial fluid gave similar results to those obtained on cattle (Table IV). Again there was no evidence of the synovial fluid cytology being influenced by the blood cytology. An eosinophilia was present in some instances and again no eosinophilic polymorphonuclear leukocytes were present in the synovial fluid.

(3) Further proof that synovial fluid cytology is not influenced by blood cytology was obtained when the same studies were repeated on normal dogs before and during the time of an experimentally produced polymorphonuclear leukocytosis. Any increase in synovial granulocytes during such a leukocytosis would be of significance because normally they are rarely present in the synovial fluid (the average being 1.7 per cent — see Table IV). As can be seen from Table V

TABLE III

*A Comparison of the Cytology of Simultaneously Obtained Bovine Blood and Synovial Fluid **

Animal No.	Material	Nucleated cells	Phagocytic cells				Non-phagocytic cells			
			Neutrophilic polymorpho-nuclear leukocytes	Monocytes	Clasmato-cytes	Unclassified phagocytes	Lympho-cytes	Synovial cells	Unclassified cells	Eosinophilic polymorpho-nuclear leukocytes
1	Blood	per cmm.	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
	S. F.	4800 130	48 0	4 60	.. 4	.. 2	46 26	.. 8	0 0	2 0
2	Blood	6000	66	2	30	..	0	2
	S. F.	170	0	52	6	6	32	4	0	0
3	Blood	7100	10	6	76	..	0	8
	S. F.	115	2	74	2	8	10	2	0	0
4	Blood	4200	18	10	66	..	0	6
	S. F.	105	0	44	6	0	48	2	0	0
5	Blood	5000	28	4	60	..	0	8
	S. F.	180	2	48	8	2	40	0	0	0
6	Blood	5100	22	6	67	..	0	4
	S. F.	120	0	60	4	4	30	2	0	0
7	Blood	10800	26	4	68	..	0	2
	S. F.	220	0	28	0	0	72	0	0	0
8	Blood	6700	42	6	40	..	0	12
	S. F.	60	0	46	4	4	40	2	2	0

* The synovial fluid was obtained from the astragalotibial joints.

TABLE IV

A Comparison of Simultaneously Obtained Canine Blood and Synovial Fluid

21 Comparisons of ...

Dog No.	Source	Totals			Phagocytic cells					Non-phagocytic cells			
		Nucleated cells	•Erythrocytes	Dead cells seen in counting room nucleated cells	Poly-morpho-nuclear leukocytes	Mono-cytes	Clas-mato-cytes	Unclas-sified phago-cytes	Lympho-cytes	Synovial cells	Unclas-sified	Eosinophilic polymorpho-nuclear leukocytes	
		per cmm.	per cmm.		per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	
1.....	Blood	11600	59	12	27	2	
	Right knee	327	155	3	0	72	4	0	21	3	0	0	
2.....	Blood	9500	76	6	18	..	0	0	
	Right knee	...	++	0	2	56	8	8	22	4	0	0	
3.....	Blood	11700	55	9	36	..	0	0	
	Left knee	1450	++	0	0	76	0	0	22	2	0	0	
4.....	Blood	15100	90	7	3	..	0	0	
	Right knee	1260	1080	0	1	90	0	0	7	2	0	0	
5.....	Blood	14500	86	9	5	..	0	0	
	Right knee	1080	360	0	7	66	6	5	8	8	0	0	
6.....	Blood	8100	71	2	24	..	0	3	
	Left knee	450	125	2	0	68	4	3	16	9	0	0	
7.....	Blood	19600	75	3	13	..	0	9	
	Right knee	1175	105	2	2	66	6	4	19	3	0	0	
	Left knee	1360	45	3	1	62	4	6	26	1	0	0	
8.....	Blood	13000	60	4	36	..	0	0	
	Right knee	825	250	0	0	69	3	0	21	7	0	0	
	Left knee	1115	160	0	0	58	2	0	36	4	0	0	
9.....	Blood	11400	83	5	6	..	0	6	
	Right knee	...	+	0	2	76	12	2	4	4	0	0	
	Left knee	565	45	0	0	66	10	6	12	6	0	0	
10.....	Blood	11600	58	10	25	..	0	7	
	Right knee	...	+	0	0	64	12	14	2	8	0	0	
	Left knee	995	400	0	0	70	20	0	4	6	0	0	
Maximum...	Blood	19600	90	12	36	..	0	9	
	Synovia	1450	...	3	7	90	20	14	36	9	0	0	
Minimum...	Blood	8100	55	2	3	..	0	0	
	Synovia	327	...	0	0	56	0	0	2	1	0	0	
Average	Blood	11610	...	0	71.3	5.7	19.3	..	0	2.7	
	Synovia	963.8	...	0.7	1.7	68.5	6.5	3.4	15.7	4.8	0	0	

* When erythrocytes were too numerous to count the number present is represented by + signs.
All differential cell counts of blood were made from fixed smears stained with Wright's stain.

TABLE V

*A Comparison of the Cytology of Simultaneously Obtained Canine Blood and Synovial Fluid Before and During an Experimentally Produced Leukocytosis**

Dog No.	Time cell counts were made	Source	Totals			Phagocytic cells					Non-phagocytic cells			
			Nucleated cells	Erythrocytes	Dead cells seen in counting 100 nucleated cells	Polymorpho-nuclear leuko-cytes	Monocytes	Clasmato-cytes	Unclassified phagocytes	Lymphocytes	Synovial cells	Unclassified non-phagocytes	Rosinophilic polymorpho-nuclear leuko-cytes	
I	(1) Before injection of sodium nucleinate	Blood	per cumm. 9300	per cumm.	67	8	18	7	
	(2) Eight hours after injection of sodium nucleinate	Right knee	...	+++	0	6	81	3	2	2	6	0	0	
		Blood	19600	...	0	95	4	1	..	0	0	
II		Left knee	990	155	4	5	76	2	4	5	8	0	0	
	(1) Before injection of sodium nucleinate	Blood	13950	...	0	77	10	10	..	0	3	
		Left knee	...	+++	0	15	66	6	3	9	1	0	0	
	(2) Fourth day of leukocytosis varying from 16 to 20,000 with 84 to 92 per cent polymorphonuclear leukocytes	Blood	19400	...	0	84.3	11.3	3	..	0	1.3	
		Right knee	1625	40	0.5	0.5	48	6.5	2	39.5	3	0.5	0	
III		Left knee	1620	++	1	0	53	0	2	36	8	1	0	
	(1) Before injection of sodium nucleinate	Blood	8400	...	0	79	4	17	..	0	0	
		Left knee	810	610	0	0	69	11	0	15	5	0	0	
	(2) Fourth day of leukocytosis varying from 14 to 23,000 with 86 to 88 per cent polymorphonuclear leukocytes	Blood	23200	...	0	87	4	7	..	0	2	
		Right knee	980	++	0	0	68	10	8	6	8	0	0	
		Left knee	1410	40	1	0	74	13	1	7	5	0	0	

* The experimental leukocytosis was produced by the injection of sodium nucleinate intravenously. In Dog I a single dose of 12 gm. was given whereas, in Dogs II and III, 2 to 6 gm. were administered each day for 4 days.

no increase in synovial fluid granulocytes occurred, whether the increased number of circulating polymorphonuclear leukocytes was of short duration (8 hours in Dog I) or of many hours duration (96 hours in Dogs II and III). Again, no eosinophilic polymorphonuclear leukocytes were demonstrable in the synovial fluid. In Dog II the number of synovial fluid lymphocytes was greatly in excess of the blood lymphocytes.

From these last three experiments it would seem that one is justified in concluding that there is no evidence to support the theory that synovial fluid cytology is a reflection of the blood cytology. The findings in the 3 pathological fluids previously cited must have been due to other causes, such as parasitic invasion of the periarticular structures in the case of the cattle and subperiosteal leukemic infiltration near the joint margins in the case of the human.

The data contained in Tables II to V inclusive offer no satisfactory explanation for the wide variations noted in average cell percentages in normal bovine synovial fluid, particularly those observed in the fluid obtained from the astragalotibial joint. The presence of a regularly occurring cartilage defect in the carpometacarpal joint and in consequence more cellular débris to be removed would seem sufficient stimulus for an increase in the total number of nucleated cells and for an increased mononuclear phagocytic cellular reaction in this joint, whereas in the astragalotibial joint no such regularly occurring pathological lesion is demonstrable and therefore there is less need for an increase in this type of phagocytic cell. The variations in phagocytic and non-phagocytic cell percentages found in the astragalotibial joint fluid probably do represent the cellular variations which can take place in any so-called normal joint. Therefore, these variations probably represent cellular reactions to the average every day insults such as wear and tear, minor trauma or any irritant, any one of which all joints must be subjected to from time to time. In other words, the cytology of normal synovial fluid is dependent in part upon what intra-articular insults have occurred. For instance, the intra-articular injection of a mild irritant such as normal saline results in a marked increase in polymorphonuclear leukocytes, only to be largely replaced in a few days by mononuclear phagocytes and later by an increase in the average number of lymphocytes.^{8, 9} Any joint subjected to unusual or constant use will show considerable evidence of wear and tear manifested by intra-articular degenerative

TABLE VI

Showing the Average Cellular Variations in the Synovial Fluid of Different Species

Animal	Joint	No. fluids examined	Nucleated cells	Poly-morpho-nuclear leukocytes	Mono-cytes	Clasmatocytes	Unclas-sified phagocytes	Lympho-cytes	Synovial cells	Unclasi-fied non-phagocytes	Total phagocytes	Total non-phagocytes
Cow	Astragalotibial	25										
	Maximum		575	8	54	36	16	62	6	4	92	64
	Minimum		55	0	12	0	0	5	0	0	36	8
	*Average		181.8	2.2	36.4	15	3.9	40.1	1.2	1.2	57.5	42.5
Cow	Carpometacarpal	12										
	Maximum		555	4	80	14	8	44	6	2	88	46
	Minimum		100	0	42	0	0	8	0	0	54	12
	*Average		213.3	1.2	63	7.2	3	23	1.7	1	74.3	25.7
Dog	Knee	14										
	Maximum		1450	7	90	20	14	36	9	0	92	40
	Minimum		327	0	56	0	0	2	1	0	60	8
	Average		963.8	1.7	68.5	6.5	3.4	15.7	4.8	0	82.4	17.6
Rabbit	Knee	9										
	Maximum		330	15	77	26	22	6	6	4	100	12
	Minimum		140	0	48	2	8	0	0	0	88	0
	Average		242.5	2.2	65.5	12.7	13	1.5	4.2	0.7	93.5	6.5

* The average cell percentages for the astragalotibial and carpometacarpal joint fluid are taken from Tables I and II respectively and represent the differential cell counts done before adding graphite.

joint changes.¹⁰ The same type of intra-articular joint change results from the wear and tear of increasing age and in consequence marked changes are demonstrable in most individuals past the fourth decade of life, even though they may never have complained of joint symptoms or joint disease.¹¹ Therefore, it is apparent that the amount of particulate matter and cellular débris present in a joint may vary considerably from time to time and in consequence the total number of synovial fluid nucleated cells, particularly the mononuclear type of phagocytic cells, must vary also. The minor insults that a joint is subjected to evidently do not cause sufficient intra-articular reaction to result in an increase in polymorphonuclear leukocytes.

A word of warning may be given to anyone interested in studying normal synovial fluid cytology, namely that there are certain species differences which one must consider if he wishes to compare the findings of one species with those of another. These differences concern the total number of nucleated cells as well as the percentage number of any one type of cell. These are best illustrated in the short summary table given below.

The above mentioned data should aid materially in interpreting the cytological variations one observes¹ in normal human synovial fluid.

SUMMARY AND CONCLUSIONS

1. A study of the cytology of the synovial fluid from the astragalotibial and carpometacarpal joints of young beef cattle showed variations in the total number of nucleated cells and in individual cell types. The widest variations in cell types were observed in the astragalotibial joint fluids.

2. The phagocytic cells (practically all were of the mononuclear type) averaged 57 per cent in the astragalotibial joint fluid and 74 to 78 per cent in the carpometacarpal joint fluid.

3. The non-phagocytic cells (chiefly lymphocytes) averaged 40 per cent in the astragalotibial joint fluid and 20 to 23 per cent in the carpometacarpal joint fluid.

4. The variations in the total number of nucleated cells and individual cell types in these two joint fluids are best explained by the increased amount of débris in the carpometacarpal joint resulting from the articular cartilage defects present.

5. The addition of a small amount of graphite to the synovial fluid before doing the supravital differential cell count aids one in distinguishing between phagocytic and non-phagocytic cells.

6. The wide variations in individual cell types observed in the astragalotibial joint fluid are evidently within the limits of so-called normal. The "normal" figure depends upon the degree of wear and tear, minor trauma, and so on, to which the joint has recently been subjected. Evidently irritations of this grade are sufficient to increase the total number of nucleated cells and the percentage of phagocytic cells exclusive of polymorphonuclear leukocytes.

7. The total number of nucleated cells contained in synovial fluid may increase postmortem but there is very little change in the individual cell percentages.

8. The cellular constituents of normal synovial fluid are not influenced by variations of the blood cytology.

9. There is a definite species difference in the total number of nucleated cells and the percentage of individual cell types contained in normal synovial fluid.

NOTE: We wish to thank the New England Dressed Meat and Wool Company for its coöperation and generosity.

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EFFECT OF CENTRIFUGATION ON HERPETIC INTRANUCLEAR INCLUSIONS WITH A NOTE ON CYTOPLASMIC INCLUSIONS OF UNKNOWN ORIGIN IN THE RABBIT CORNEA *

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A conspicuous evidence of some virus diseases is the production of intranuclear inclusions. It has not been possible thus far to determine unequivocally by the usual microscopic methods whether the smallest visible granules are the virus organisms or the products of a cellular reaction to an invisible virus. The ultracentrifuge developed by Beams, Weed and Pickels¹ provides a new method of approach to these problems in that sufficient centrifugal force is attained to displace the established relationship of cellular elements, and thereby enables one to make comparisons of this displacement with chromatin and nuclear sap, substances about which something is already known.

METHOD

Herpes virus, H. F. strain, originally obtained from the Rockefeller Institute for Medical Research through the kindness of Dr. T. M. Rivers, was employed. Fresh brain of rabbits previously inoculated intracerebrally was triturated and applied to the scratched cornea of rabbits. About 30 to 48 hours later the infected eye was removed and the cornea cut into three pieces, one of which was placed in a duralumin rotor of the ultracentrifuge and whirled at 65 to 70 pounds air pressure for 45 to 60 minutes. The remaining two pieces were fixed in Zenker's acetic fixing fluid, one before centrifugation began and the other after it had been completed. Autolytic effects upon the corneal tissues due to time alone were thus differentiated from centrifugation effects. Corneal tissues, not infected, were treated in the same way. Part of this control material was scratched with a needle about 30 hours previous to removal in order to distinguish factors involved in regeneration from those due to infection

* Received for publication July 5, 1935.

The preparation of the manuscript was completed while one of us (A.M.L.) was at the Anatomical Laboratory, Washington University, St. Louis, Mo.

when these tissues were centrifuged. Delafield's hematoxylin with eosin, Ehrlich's hematoxylin with triosin, and Giemsa stains were used.

OBSERVATIONS

The cornea is from 4 to 5 cells thick: the basal layer contains large oval nuclei with the long axes perpendicular to the basement membrane, the overlying nuclei are smaller and denser and their long axes parallel to the surface.

Normal Epithelial Cells: When normal corneal epithelium or regenerating corneal cells are centrifuged, both nuclei and cytoplasm are modified to some extent. The cytoplasm balloons centripetally in the outer two or three rows of flattened cells (Fig. 6). The position of the nucleus in the cytoplasm does not change. The chromatin of the nucleus becomes massed toward the centrifugal pole and the opposite end becomes correspondingly free of it but is filled with clear, homogeneous, non-staining nucleoplasm.

No difference was noted in the reaction of nuclei in normal uninjured epithelium and in that undergoing regeneration.

Infected Epithelial Cells: The infected corneal cell nuclei have the appearance represented in Figure 3. The granular inclusion body lies in the center of a nucleus and is usually surrounded by a clear halo. The chromatin is closely packed against the nuclear membrane.

The translocation of chromatin materials by centrifugation in the normal nucleus is relatively slight (Figs. 6 and 7A), but is readily accomplished in the infected nucleus (Figs. 1, 2, and 7B). In the latter the chromatin, as in the normal nucleus, moves toward the centrifugal pole, the fluid, non-staining material forms a narrow middle zone and the inclusion body presses centripetally against the nuclear membrane.

It was sought to determine under high magnification whether the chromatin passes around or through the inclusion body. Most frequently it follows the contour of the nuclear membrane but occasionally, as in Figure 7B, it passes through the mass of inclusion granules and in some cases numerous chromatin particles were found among the granules.

Occasionally the nuclear membrane breaks (Fig. 7C), in which case the granules of the inclusion become slightly separated and pass into the cytoplasm in a centripetal direction. The resistance of the

nuclear membrane against rupture has been noted by Nĕmec,² and Luyet and Ernst^{3,4} in plant cells.

Cytoplasmic Inclusions of Unknown Origin: Spherical, cytoplasmic inclusion bodies were found in the epithelial cells of 8 out of 10 corneas examined; the 2 negative cases are doubtful because sufficient material was not available. They are present in control and virus-infected tissues, in both uncentrifuged and in centrifuged portions. Their distribution is not uniform; the bodies are limited to small areas, irregularly scattered, and in any one location the number of inclusions varies greatly. They are present usually in the cells lying above the basal layer and extend into that layer only when very numerous (Fig. 4).

The corneal inclusion body lies closely associated with the nucleus and profoundly changes its shape and character (Figs. 4 and 5). It is bounded by a distinct membrane, inside of which are small, poorly defined non-refractile granules which form part of a reticular network. The granules and reticulum stain with acid dyes but somewhat more lightly than the adjacent cytoplasm. A clear, non-staining fluid fills the interstices.

The inclusion bodies vary in size from $1.5\ \mu$ to $10.5\ \mu$. Each one, irrespective of size, lies within the clear, non-staining area which separates nucleus from cytoplasm.

The origin of the inclusion bodies is not known; the smallest bodies thus far identified already occupy the same close proximity to the nucleus as the larger ones (Fig. 5A). Their number in a cell varies from one to four and they lie on either side or end of the nucleus. The effect on the nucleus is the same in each case, namely, a depression of its wall within which the inclusion body extends. The small spheres produce only a slight indentation (Figs. 5A and 5B), where those which are larger in proportion to the nucleus (Figs. 5C and 4A) may nearly constrict it in two parts or may flatten the nucleus until it finally appears as a deeply staining, pyknotic crescent on one side of the inclusion; or in the case of two or more bodies it may be crowded into a small, crumpled, irregularly shaped mass compressed into the space available between the spheres (Fig. 4B). Due to the plane of section the cytoplasmic inclusion sometimes resembles an intranuclear inclusion (Fig. 5D). When they are large and numerous the cytoplasm and the tissue as a whole, as well as the nuclei, appear abnormal.

Effect of Centrifugation on the Corneal Cytoplasmic Inclusion Bodies: Whirling the corneal tissue at 65 to 70 pounds pressure for from 45 minutes to an hour, which is sufficient to displace the intranuclear inclusions of herpes, does not displace the cytoplasmic inclusions in relation either to the rest of the cytoplasm or to the nucleus.

DISCUSSION

Centrifugation separates cellular elements into strata and thereby aids in an analysis of heterogeneous structures. The herpetic intranuclear inclusion is lighter than the nuclear sap or the chromatin; therefore, if the inclusion body arose by abnormal multiplication of elements, which were already present in the normal nucleus, one might expect that this material, being the lightest in the cell, would appear at the centripetal pole of the centrifuged normal nucleus. No such inclusion body precursor is revealed. Its absence, of course, does not preclude the possibility that some element, either chromatin or nuclear sap, is transformed into inclusion body material by the action of the virus, these materials being consumed in the process and consequently decreasing in amount. Evidence for this point of view is weakened when by centrifugation it is apparent that the supposed decrease in chromatin is not as great as it seems to be when margined (Figs. 1, 2 and 7). Although no quantitative measure has been devised, it appears from the slides and illustrations that when all the chromatin of the infected nucleus is brought to one pole the amount is only somewhat less than that present in centrifuged normal nuclei. Comparison, of course, must be made between nuclei at corresponding levels in the epithelium.

Possible physical antagonism between chromatin and inclusion material, suggested by the tendency of basophilic substance to marginate soon after the appearance of inclusion granules, finds some support under conditions of centrifugation. Examples have been noted in which the chromatin mingles with the inclusion material while passing to the centrifugal pole. However, high magnification of one of these, Figure 7B, shows a narrow clear column surrounding the line of migrating chromatin separating it from the adjacent inclusion material, indicating that a repellent force between the two substances is still operating even when they are forcibly mingled together.

The physical constitution of the infected and normal nuclei is dif-

ferent in that the same centrifugal force readily brings all the chromatin to one pole in the inclusion-bearing cells and only partially pulls the chromatin of normal cells away from the centripetal end of the nucleus. In most instances the chromatin of the normal nucleus is not moved at all. A specific gravity of chromatin greater than that of the nuclear sap has been found in both plant and animal cells (Němec,² Beams and King,^{5,6,7} Luyet and Ernst,^{3,4} and Scott⁸). A wide variation in the amount of centrifugal force necessary to produce the translocation of materials agrees with observations by Beams and King⁷ on nerve cells.

The inclusion body itself shows no stratification of materials or separation of granules from the fluid which surrounds them. This is to be expected since the granules are apparently already packed as closely as possible.

Earlier characterization of intranuclear inclusions of virus origin by their acidophilic staining affinities indicated a difference from the basophilic staining chromatin. The existence of a difference between the two materials has been further emphasized by the Feulgen thymonucleic acid reaction which is positive for mammalian chromatin and is negative for inclusions of herpes,⁹ virus III,⁹ and yellow fever.¹⁰ Likewise, following microincineration, little or usually no mineral ash remains in the inclusions of submaxillary gland virus of guinea pigs,¹¹ of yellow fever,¹² or of well developed herpetic inclusions,¹³ whereas an abundant ash is present from the chromatin. Centrifugation reveals, likewise, a difference, namely that the inclusion material is lighter than any part of the normal nucleus; all of which suggests that the intranuclear inclusion is probably not derived from chromatin.

The corneal cytoplasmic inclusions described do not arise as a result of herpetic infection since they occur equally often in apparently normal rabbits. Their high incidence makes it probable that they persist for relatively long periods of time. Whether they are pathological is not certain but when by their great number and size they distort the nucleus and cytoplasm, as shown in Figure 4, it is difficult to regard them as normal cell structures. The distinct membrane which surrounds each sphere separates it from the adjacent cytoplasm, but the internal structure is not greatly different. Their principal effect is on the nucleus but the resulting modification on its shape and character is not proof of a detrimental influence. The in-

dentation of the nucleus calls to mind a similar reaction by vaccine virus inclusions in fixed and stained preparations. The shrinkage space which surrounds the nucleus and its apposed corneal cytoplasmic inclusion body may be indicative of a perinuclear material different from that existing in the remainder of the cytoplasm. This agrees with a suggestion by Cowdry,¹⁴ in his study of vaccine virus inclusions, that the "appearance is strongly suggestive of shrinkage at interfaces between fluids of different consistency and composition."

No leukocytic infiltration occurs in the region where the corneal inclusions occur. Leukocytic infiltration in vaccinia is variable; in some cases it may, likewise, be absent from the region in which the inclusion bodies are found.

SUMMARY AND CONCLUSIONS

1. Centrifugation of rabbit cornea inoculated with herpes virus concentrates the margined chromatin to the centrifugal pole, causes the nuclear sap to form a clear stratum across the middle of the nucleus, and moves the inclusion to the centripetal pole.

2. The concentrated margined chromatin, when brought to one pole, appears to be slightly less in amount than the chromatin of the normal nucleus.

3. Antagonistic forces between chromatin and inclusion body, expressed by the phenomenon of margination, is still operating when chromatin is forced by centrifugation through the inclusion body, in that the chromatin in passing through is separated from the granules by a distinct space.

4. Centrifugation of normal nuclei moves the chromatin slightly toward the centrifugal pole and the nuclear sap to the opposite pole. No substance is concentrated which by its relative specific gravity or staining can be regarded as the direct antecedent of the granules present in the herpetic inclusion body.

5. Cytoplasmic inclusions of unknown origin were found in the epithelial cells of both normal and infected rabbit corneas. They indent the nuclear walls and when large, the nuclei become crescentic or compressed to a small, irregularly shaped body. The pathological nature of the corneal cytoplasmic inclusion body is not yet established.

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DESCRIPTION OF PLATES

The photomicrographs were taken by F. W. Kent. The drawings* were made by Miss G. L. Larsen. The arrow in Figures 1, 2, 6 and 7 points to the centrifugal pole.

PLATE 127

FIGS. 1 and 2. Photographs of the same group of corneal cells in which the intranuclear inclusions of herpes have been displaced centripetally by centrifuging for 45 minutes at approximately 494,000 times gravity. $\times 850$ and $\times 2640$.

* We are indebted to the Graduate School of the University of Iowa for funds for the preparation of the drawings.



PLATE 128

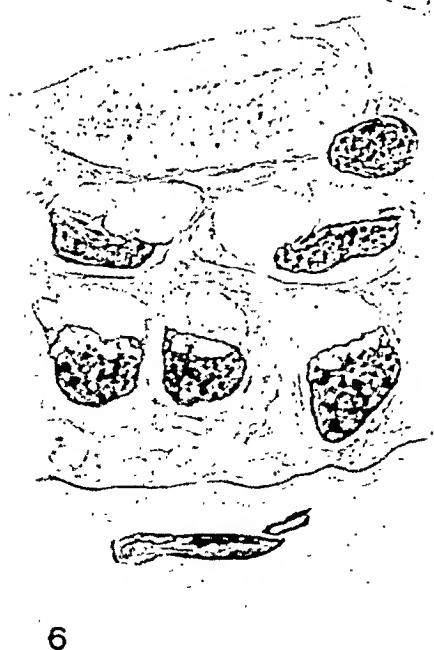
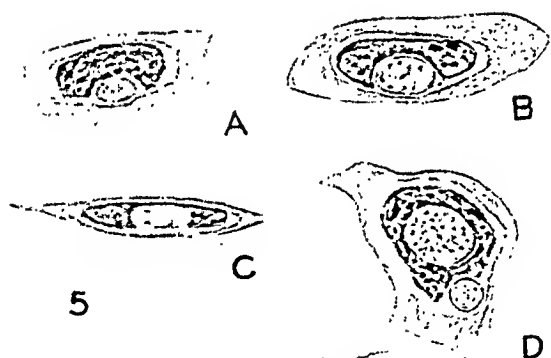
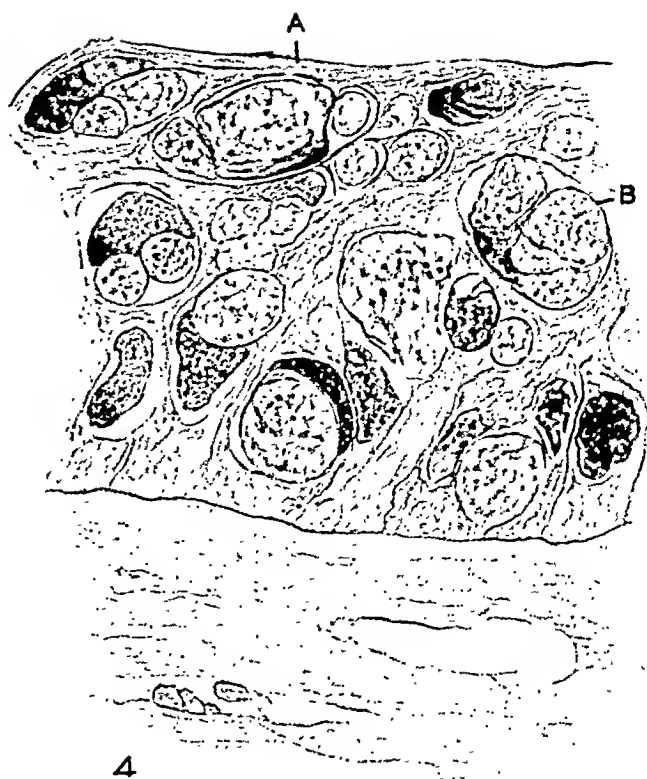
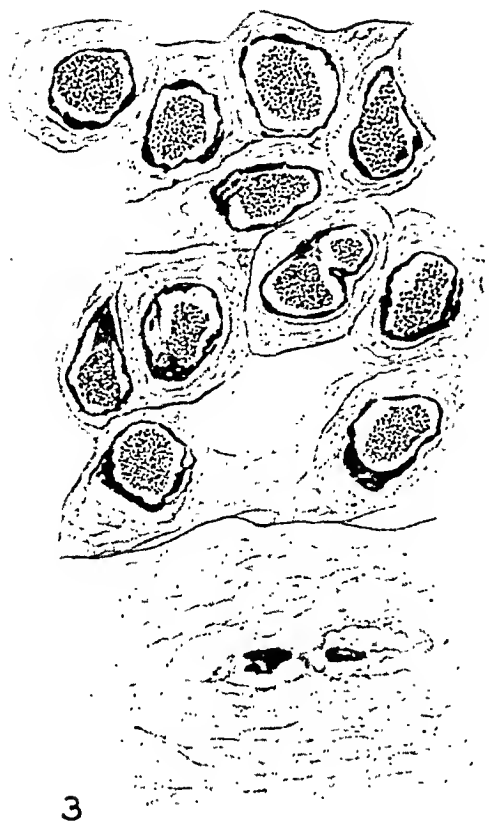
FIG. 3. A control section from the same cornea as shown in Figures 1, 2 and 7, showing cells containing intranuclear inclusions of herpes. $\times 1300$.

FIG. 4. A region of cornea containing numerous cytoplasmic inclusion bodies which are of unknown origin. $\times 1300$.

FIG. 5. Four cells showing the relation of cytoplasmic inclusions to the nuclei. $\times 1300$.

FIG. 6. Normal corneal cells after centrifuging for one hour at approximately 494,000 times gravity. $\times 1300$.

FIG. 7. Drawing of the same group of cells illustrated in Figures 1 and 2. $\times 1300$.



Herpetic Intranuclear Inclusions

PRIMARY AMYLOIDOSIS LIMITED TO TISSUE OF MESODERMAL ORIGIN *

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DEDICATED TO PROFESSOR ANTON GHON OF THE GERMAN UNIVERSITY IN PRAGUE
IN HONOR OF HIS SEVENTIETH BIRTHDAY

Amyloidosis secondary to various chronic diseases is relatively common and well known, in contrast with the type regarded as atypical in which the condition appears to be primary. About 35 cases of the latter type have been reported, chiefly from German sources. Of these there are 17 which resemble the type described here. Most of them were studied and reported within the past 6 years.

The following case is of unusual interest since it is apparently the first reported to be diagnosed clinically and because of the generalized and extensive involvement of the smooth musculature of the cardiovascular system, the mesodermal structure of the lung and serous membranes.

REPORT OF CASE

Clinical History: In January, 1933, a female, 41 years of age, first noted intermittent pains in the shoulders and arms, especially at night, aching pains in the legs after walking, and later general weakness. A goiter appeared about the same time. In May, 1933, sore throat developed with cervical adenitis, fever and pain, which necessitated 2 weeks stay in bed. Because the fever persisted the patient was sent to a hospital by her physician. An X-ray examination of the chest, various agglutination tests and a Mantoux test all gave negative results. There were, however, albumin (1+) and a few red cells in the urine, and the hemoglobin was said to be 60 per cent. During the summer and autumn of 1933 weakness persisted, edema of the legs and ankles was noted toward evening and there was continual low grade fever, tachycardia and malaise. The record is then blank until July, 1934, when submental swelling was noted. The menstrual periods had been scanty during the previous year and ceased in September, 1934. In October the tongue was noted to be thick and red and felt as if blistered. Weakness, dyspnea on exertion, loss of weight, dysphonia and dysphagia increased. The tongue continued to increase in size and the skin over the chin became hard. The condition was regarded as malignant. Because of the progressive nature of the disease and loss of 31 pounds in weight, the patient entered the University Hospital in January, 1935.

* Received for publication July 1, 1935.

On physical examination the patient evidently had lost much weight and there was dyspnea and a slight icteric tinge of the skin. The veins of the neck were distended and the skin over the chin was thickened, waxy and fixed to the underlying tissues. Firm, large, tumor-like masses were present under the mandible and the posterior cervical lymph nodes were enlarged and firm. The thyroid gland was diffusely enlarged. The tongue was thickened to about twice the normal size, with a red smooth surface indented by the teeth. There was evidence of pleural effusion and râles were present in the left lung. The heart appeared to be normal. The systolic blood pressure was 128 and the diastolic 78. The liver was palpable but apparently normal. Examination of the pelvis revealed hard, leathery induration of the clitoris and vulva, thickened, firm vaginal walls, a hard cervix and an enlarged firm uterus with irregular contour and restricted mobility.

Laboratory Data: Urine normal, no Bence-Jones protein; red cells 5 million per cmm., hemoglobin 78 per cent, leukocytes 4900 per cmm. with 77 per cent polymorphonuclear cells. Blood Wassermann reaction negative; basal metabolic rate +12 per cent; sedimentation rate first hour 48 mm., second hour 77 mm. The Congo red test was negative as 30 per cent of 125 mg. were removed from the blood in 1 hour. Plasma proteins per 100 cc.: fibrinogen 0.52 gm., euglobulin 0.38 gm., pseudoglobulin 1.9 gm. and albumin 3.52 gm., giving a total of 6.32 gm. Mantoux test 0.1 mg. positive.

X-ray examination (Fig. 1) revealed pleural effusion in both bases, more in the left, and a diffuse infiltration throughout both lungs following the broncho-vascular trees and suggesting some type of pulmonary congestion of rather extreme degree or an infiltrating process. There was a large mass in the region of the right hilum. The femurs, pelvis, humeri and skull were negative.

Clinical Course: The temperature ranged between 37.2° C. and 37.8° C. (99° F. to 100° F.) until January 17th when peritonitis developed, characterized by chilly sensations, emesis, abdominal distention, fever of 39.7° C. (103.4° F.) and 56,000 leukocytes per cmm. Death occurred 6 days later.

A diagnosis of amyloid disease of the tongue, skin and probably of the genitalia and mediastinum, with terminal peritonitis, was made. The diagnosis was confirmed by biopsy of the tongue, skin and vaginal wall. Previous experience and the privilege of studying another case with an amyloid tongue which was subsequently reported by Michelson and Lynch¹ led to the clinical recognition of the case here reported.

POSTMORTEM EXAMINATION

The right pleural cavity contained a liter of clear fluid, the left was partly obliterated. There were no adhesions in the pericardial sac. The heart weighed 360 gm. The left auricular wall when cut did not collapse and appeared as if frozen or infiltrated with carcinoma. It was rubbery, and when bent sprang back into position. The right auricle was less involved, while the ventricles, chordae tendineae and the root of the aorta appeared to be normal in color and consistence.

The right lung weighed 1160 gm., the left 550 gm. There were edema and patchy lobular atelectasis, most marked on the left. The consistence was unusual and suggested that of frozen lungs; they were firm, heavy and did not collapse. Apparently they were not consolidated and on the cut surface the alveoli remained open. The pulmonary veins were from 2 to 4 mm. thick, stiff and fixed to the mediastinal structures. The entire mediastinum was firm, hard and fixed. The heart was held firmly in the mass by the greatly thickened vessels. The trachea and aorta were similarly fused together as if by fibrous tissue, although nothing was found to suggest mediastinitis. The lymph nodes were normal. The wall of the lower portion of the esophagus was thickened, stiff and did not collapse. There were no abnormal changes in the aorta. The tongue appeared as described clinically, with its epithelial surface intact. The submaxillary lymph nodes were not enlarged. Enlargement of the thyroid gland (150 gm.) was due to multiple adenomas.

The peritoneal cavity showed generalized peritonitis with injected serous surfaces and turbid fluid containing fibrin and hemolytic streptococci. The spleen (325 gm.) was uniformly dark in color, resembling the type encountered in long-standing passive congestion. A portion tested with iodine and sulphuric acid failed to give the reaction characteristic of amyloid substance. The liver (2250 gm.) was not remarkable and failed to give the amyloid reaction.

The kidneys (490 gm.) showed "flea-bitten" surfaces characteristic of acute glomerulonephritis or of embolic nephritis. The hemorrhagic appearance was also found on the cut surfaces. The right ureter was normal but the lower third of the left was thickened. Its wall and adjacent tissues, including the ovarian vein, were involved in an acute inflammatory reaction. This process extended through the left broad ligament into the region of the cervix. Edema, small pockets of pus and the presence of many hemolytic streptococci indicated that this was a terminal infection.

The uterus was enlarged to the size of a 2 months gravid uterus, the muscle averaging 4 to 5 cm. in thickness. It cut with increased resistance. Both cervix and uterus were hard. The vaginal wall was remarkably thickened and difficult to cut. Its appearance and consistence resembled that of a malignant infiltration. The ovaries were hard, sclerotic and atrophic. There was an acute pyophlebitis of the left ovarian vein.

No important changes were noted in the stomach, bowel, pancreas, adrenals, urinary and gall-bladder, brain or meninges.

Anatomical Diagnoses: Amyloidosis of the tongue, heart, lungs, esophagus and pelvic organs; acute generalized peritonitis; pulmonary edema and atelectasis; pleural effusion; phlebitis of the left ovarian vein; glomerulonephritis or embolic nephritis; and adenomas of the thyroid gland.

MICROSCOPIC EXAMINATION

The sections were stained with hematoxylin and eosin, Mallory's connective tissue stain, azocarmine, Van Gieson's stain, methyl violet, cresyl violet, Congo red, and iodine followed by sulphuric acid. Unless stated otherwise, the descriptions are based on the appearance of sections stained with hematoxylin and eosin.

Except for the changes incident to the terminal infection, namely phlebitis of the cervical, parametrial and ovarian veins and peritonitis, the findings centered on the amyloid deposits and connective tissue changes. The blood vessels throughout the body showed marked deposition of amyloid substance. The increase in size of the vessel walls and the resulting distortion of the tissues made it difficult in places, especially in the lung, to distinguish between veins and arteries. In the cervix and tongue the distinction was fairly clear; in the liver, kidneys and heart the identification was easy. As far as could be determined, the changes observed were entirely limited to the arteries, principally the small and medium sized vessels. This vascular involvement was present in the subcutaneous tissue, fat, loose areolar tissue, peripheral muscle, thyroid, salivary glands, larynx, lungs, heart, esophagus, small bowel, liver, spleen, adrenals, kidneys, uterus, cervix, ovaries, vagina, bladder and pancreas. The arterioles, such as those entering the glomeruli of the kidneys, and the capillary vessels throughout the tissues were normal. In the largest vessels, such as the aorta and renal arteries, only the vasa vasorum were infiltrated. The degree of involvement was not uniform in all the vessels. Those of the tongue, cervix, vagina and submucosa of the small bowel were much more extensively affected than those of other tissues.

In detail, the wall of the involved vessel was enormously thickened (Figs. 2, 3 and 4), three fairly definite layers could be distinguished — a loose cellular internal zone, a compact relatively acellular or

hyaline-like medial layer, and a fibrous, compressed peripheral layer. The internal zone was made up of a loose network of fibroblasts with a layer of endothelium separating it from the lumen. The peripheral layer appeared as though it were the remains of the adventitia compressed by a greatly enlarged media. In the middle layer only a few irregularly grouped nuclei were seen. Among these nuclei were masses of homogeneous, eosin-stained substance. Further analysis of these tissue changes was made by means of the differential stains as described below.

The size of the lumens was difficult to estimate. The compression of the adventitia and the loss of substance between the vessels indicated that the vessels enlarged peripherally. An estimation of the ratio of the diameter of the lumen to that of the entire vessel, therefore, was of no value. The lumen was seldom occluded. The absence of patchy atrophy of the kidney such as is seen in sclerotic disease of arteries also indicated that very little, if any, occlusion took place.

In addition to the arterial changes there were connective tissue changes in the tongue, esophagus, auricles and ventricles. The muscle fibers were spread apart by varying amounts of irregularly distributed material, partly fibrillar and partly homogeneous (Figs. 5 and 6). In certain places this material was in wide bands. The muscle bundles were not themselves invaded but suffered atrophy and replacement, as evidenced by loss of striations, shrinking of the bundles and, finally, complete atrophy. In the tongue and auricle wall the replacement of muscle took place in a coarse and irregular fashion; in the esophagus and ventricle it was fine and evenly distributed.

The lung manifested still another change (Fig. 7). In addition to the heavily involved blood vessels there were small bands, rounded nodules and irregular plaques of eosin-stained material within the alveolar walls. None of the deposits was large enough to fill the alveolar space. They were uniformly distributed throughout all portions examined from both lungs with scarcely a single microscopic field free from involvement. The changes accounted in part for the unusual roentgenogram of the lungs and the unusual consistence noted in gross. The bronchial mucosa, muscle and lymph nodes were not involved.

A similar eosin-stained substance was found in the serous mem-

brane of the spleen, accessory spleen and small bowel, but not in that of the examined sections from the liver, ovary, uterus, lung or heart. The thickening was uniform and regular, and was differentiated from simple, thickened fibrous peritoneum by the absence of nuclei and by the use of special stains.

The parenchyma of the liver, spleen, adrenals, pancreas and thyroid, other than the blood vessel changes, showed no significant change. The glomeruli of the kidneys showed a terminal glomerulitis, manifested by slight proliferation of the endothelium. At the periphery of several of the arteries in the cervix there were large giant cells. The nuclei of these cells were irregularly arranged in the center of the cytoplasm. No inclusions or phagocytosed granules were visible. A few small lymphocytes were scattered about the giant cells. This collection of multinucleated cells was not found in other parts of the body. It may have represented a foreign body reaction to the presence of the amyloid.

SPECIAL STAINING METHODS

The similarity of staining reactions of the substance, as found in the arterial walls and elsewhere, permits inclusive description of the study. Of the stains used, Mallory's connective tissue stain proved to be of most value. It was evident that the deposits were composed of two different materials. One was an irregularly arranged, rough, coarse substance which stained like typical amyloid substance with Mallory's and Van Gieson's stains, eosin, cresyl violet and methyl violet, but failed to stain with Congo red or with iodine and sulphuric acid. This amyloid substance appeared to be embedded in a different and more delicate appearing substance, which stained less deeply with eosin and did not give the amyloid reaction (Fig. 5). The amyloid substance was found only within the largest collections of the latter material. In the ventricular muscle, for example (Fig. 6), there was extensive strangulation of the fibers by fine strands of the delicate appearing substance in which only occasional faintly stained areas suggested the presence of typical amyloid substance. On the other hand, under the intima of the auricle, there were wide bands of the delicate substance and these contained numerous rough masses of amyloid substance. The atypical, delicate appearing material which formed the bulk of the deposits between the muscle bundles was regarded as "ground" substance.

Azocarmine is generally believed to stain both adult collagen and its immature or precollagenous form, whereas by Van Gieson's and Mallory's methods only the adult type is stained. The ground substance in question did not react like adult collagen in this respect. It did not retain Van Gieson's or Mallory's stain, stained faintly with eosin but, like premature collagen, was stained deep blue with azocarmine. This behavior, however, does not prove it to be precollagen. Since amyloid substance is also stained with azocarmine and eosin, one may suggest with equal right that the ground substance may be the precursor of amyloid substance. The presence in this case of amyloid in only the larger, apparently older masses of the ground substance was so constant as to suggest strongly that the latter material probably was "pre-amyloid." It is possible also that the amyloid substance itself may not be entirely mature in the usual sense since it failed to react typically with Congo red or with the iodine and sulphuric acid test. One is also led to suspect that the "ground" substance increases at the expense of the muscle fibers and, as it increases in volume, amyloid appears in the center or oldest portion. The pathogenesis of this substance appears to be fundamentally different from that found in secondary amyloidosis. In the present case the substance appeared to have been formed and deposited locally whereas in the secondary form it is deposited in organs and areas especially designed for the removal of normal or abnormal excess substances from the circulating blood. The relation of the substance to "hyalin" was widely discussed 30 years ago, but until further chemical studies are made little progress can be made in this regard.

DISCUSSION

Aside from Wichmann's ² comprehensive survey in 1893 in which amyloid disease was classified into local and generalized forms, no attempts at further classification were made until Lubarsch's ³ paper appeared in 1929. Lubarsch recognized an essential difference between various forms and grouped them into the typical or commonly recognized form and the atypical (systematized) form into which group the present case falls. Since then numerous terms have been introduced, some of which seem to confuse rather than clarify the classification. Terms such as typical, genuine, classical, visceral, generalized, orthochromatic, pericapillary, periglandular and so on

are applied to the common form of amyloid disease which often follows chronic illness. The atypical form is described as primary, unusual, idiopathic, systematized, paramyloidosis, and so on. Several separate groups are proposed to accommodate variations such as tumor-forming amyloidosis, variations in staining properties, and according to the localized distribution of amyloid substance. The terms "typical," "genuine" or "classical" are justified only to the extent that they signify the form of disease first studied and more commonly encountered. The terms "visceral," "generalized," "systematized" or "localized" may apply to either form of the disease. The adjective "unusual," or "paramyloidosis," are pointless and until more knowledge of the chemistry of amyloid protein or proteins is attained, subdivision on the basis of staining reaction is of little value. A simple clinicopathological classification is as follows:

- I. Primary amyloidosis
- II. Secondary amyloidosis
- III. Tumor-forming amyloidosis
- IV. Amyloidosis associated with multiple myeloma.

Even this proposal is not entirely satisfactory because of the frequent overlapping of characteristics. For example, von Bonsdorff's case ⁴ apparently primary with multiple amyloid tumors, resembled the type which accompanies multiple myeloma. In Gerber's ⁵ case of the "typical" form there was extensive involvement of the bone marrow. In the case reported by Michelson and Lynch,¹ presumably one of multiple myeloma, amyloid substance was distributed as in the primary form, but except for Bence-Jones proteinuria and doubtful X-ray evidence it is, however, uncertain, without autopsy evidence, if myeloma actually existed. In general, however, certain differences are quite constant.

I. *The primary form* is characterized by (a) absence of preceding disease, (b) no involvement of organs or tissue usually affected in the secondary form, (c) involvement of mesodermal tissue, cardiovascular system, gastro-intestinal tract, smooth and striated muscle and lymph nodes, (d) variation in staining reactions and (e) tendency to nodular deposits. This form is thoroughly discussed by Strauss.⁶

II. *The secondary form* usually follows chronic disease and is

characterized by large deposits, especially in the spleen, liver, kidney and adrenals, and by typical staining reactions.

III. *Tumor-forming amyloidosis* has been especially studied by von Bonsdorff. This form is characterized by the presence of small, solitary or multiple tumors in the eye, bladder, urethra, pharynx, tongue and especially in the respiratory tract. It is usually of the primary type but is distinctive enough to be grouped separately.

IV. *Amyloidosis occurring with multiple myeloma* is in a class apart. It is secondary in nature but the distribution and character of the deposits frequently resemble those of the primary form except that huge deposits may occur in the joints and elsewhere. The spleen and liver are seldom infiltrated. Small deposits are occasionally found in blood vessels of the heart, spleen, and elsewhere. Thirty-seven cases of the latter group have been surveyed by Magnus-Levy.⁷

There have been thus far about 35 cases reported which fall into Group I⁸; 17 of these resemble more or less the case reported here, while in the remainder the amyloid substance was localized to one or two organs, as in the cases reported by Beneke and Bönning, Landau, Beckert, Kann, Beneke, Königstein, Budd, Brocher and Humphreys.⁸ Picchini and Fabris' and Gottron's cases were almost identical with ours.

The clinical characteristics of cases included in Group I are fairly characteristic. The disease is apparently primary in nature, occurs in middle-life or later, and is characterized chiefly by involvement of the skin, tongue and smooth and voluntary musculature. The skin, usually of the face and neck is thickened and stiff, the tongue and involved muscles become greatly enlarged and firm. Infiltration of the intestine results in diarrhea or constipation and occasionally hemorrhage. Hemorrhages may occur into the skin and elsewhere. Involvement of the mediastinum, lungs and heart may result in dyspnea or heart failure.

Weakness and loss of weight are frequent but the blood pressure is seldom affected. Death from renal insufficiency, as occurs in the secondary form, has not been reported. The Congo red test was positive in Gottron's case but negative in von Bonsdorff's and our own.

Histologically the characteristics are striking in respect to the generalized involvement of the smooth and striated musculature, especially of the cardiovascular system, gastro-intestinal and genito-

urinary tracts, tongue and diaphragm. Involvement of the central nervous system has not been reported. The media of small or medium sized arteries is chiefly affected.

In muscle tissue amyloid deposits occur *between the fibers and in the connective tissue spaces* in the form of bands, masses or nodules. Deposits may be found in sebaceous and sweat glands, in the loose connective tissue around the aorta, in tendon sheaths, in the alveolar walls of the lung and elsewhere. With special stains the substance may react like "typical" amyloid substance, but usually atypically, weakly or not at all.

The etiology of primary amyloidosis is unknown. Strauss cited a number of associated diseases, but in practically all cases, as in our own, these appeared to be incidental or terminal. Letterer⁹ suggests the involvement of an antigen-antibody reaction but in the reverse sense as compared with the reaction presumed to exist in the secondary form of amyloidosis. According to Wichmann² and Schmidt¹⁰ the process in general resembles infiltration more than it does disintegration or degeneration, since the organs involved usually increase in size and weight without evidence of intracellular deposits. In our own case, exclusive and widespread involvement of tissue of mesodermal origin and histological evidence of the local origin of the amyloid substance strongly implicated some unknown change, perhaps in the nature of generalized metabolic perversion of tissue of this particular origin.

SUMMARY

A case of primary amyloidosis of 24 months duration is reported. After an incidental respiratory infection there was continual low-grade fever, progressive weakness, loss of weight and slight evidence of cardiac failure. Swelling of the tongue was noted 15 months later and death was caused by acute peritonitis. A clinical diagnosis of amyloidosis was made and confirmed by biopsy and autopsy. The Congo red test was negative.

The striking pathological change was amyloidosis limited to tissue of mesodermal origin, notably the smooth musculature of the medium sized arteries of all of the organs and tissues examined, the mesodermal structures in the lung and the serosal surfaces.

A simple classification of amyloid disease is presented with special reference to the type regarded as primary in nature.

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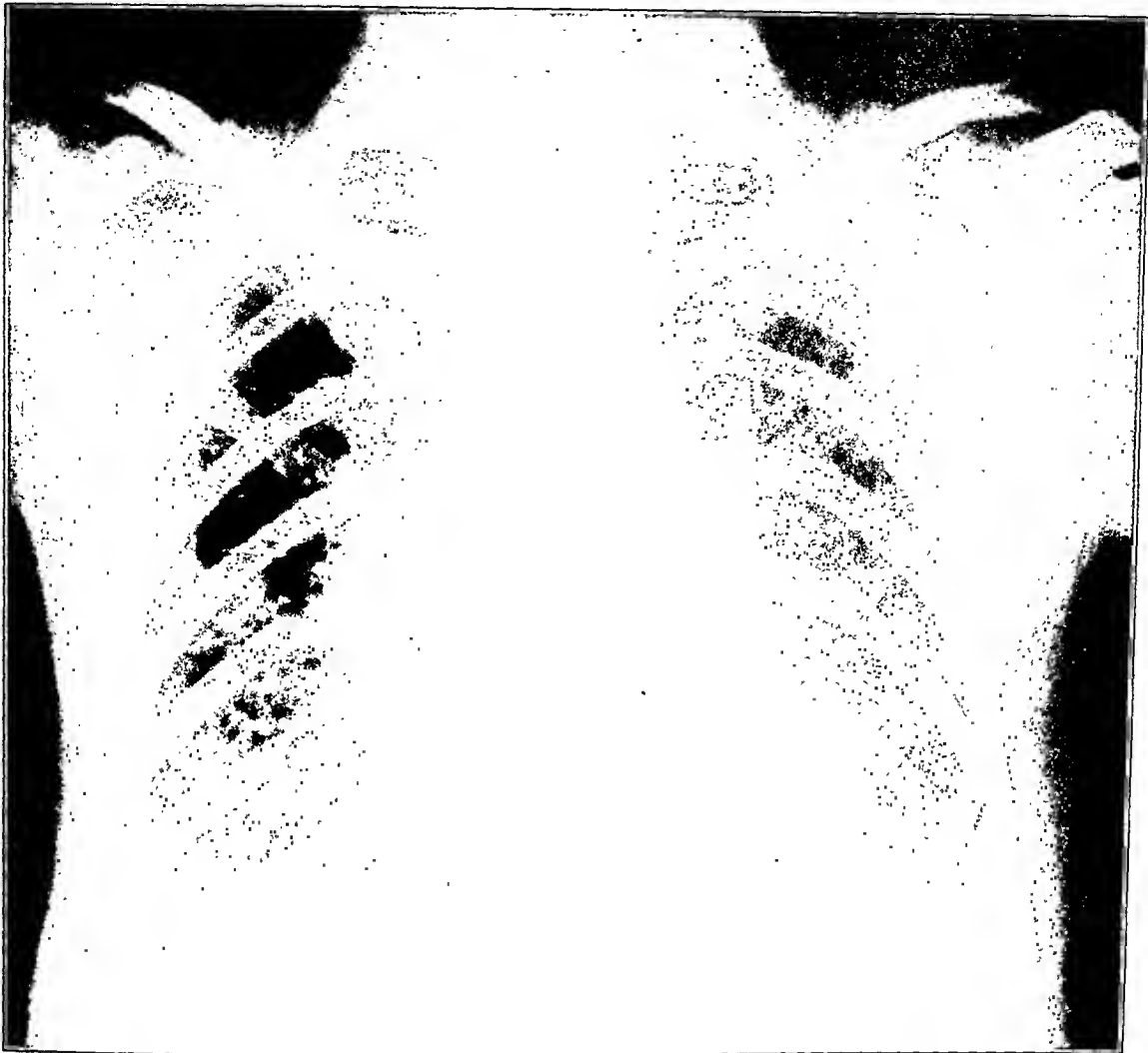
DESCRIPTION OF PLATES

PLATE 129

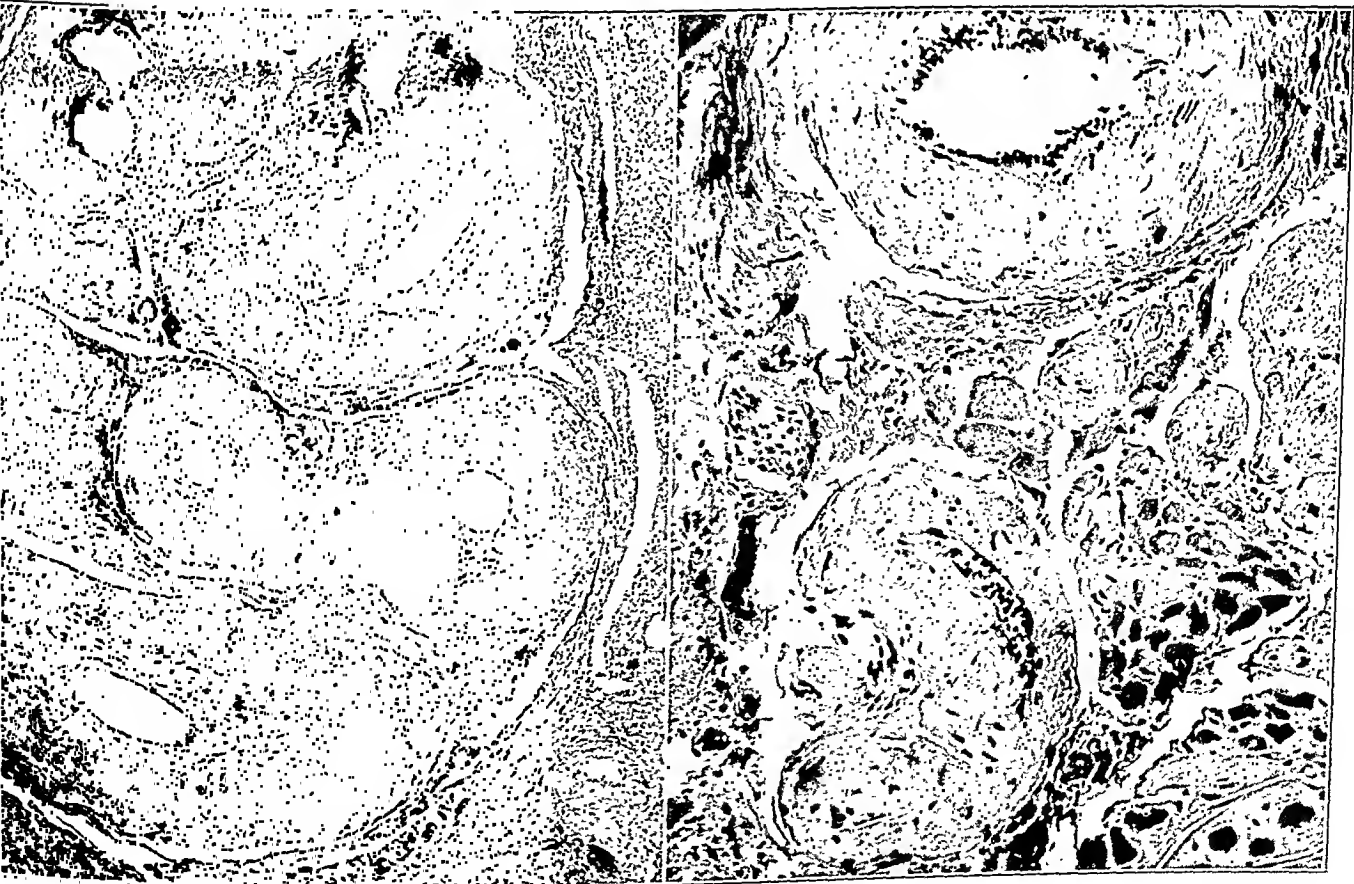
FIG. 1. Roentgenogram showing diffuse amyloid infiltration of mediastinum and lungs.

FIG. 2. Arteries of cervix. The media is enormously enlarged by amyloid substance which displaces the nuclei irregularly. The intima is a loose network of fibroblasts with a layer of endothelium. The adventitia is partly obliterated, as if by pressure. Hematoxylin-eosin preparation. $\times 150$.

FIG. 3. Arteries of tongue showing changes similar to those described in Figure 2. The muscle fibers are spread apart by amyloid.



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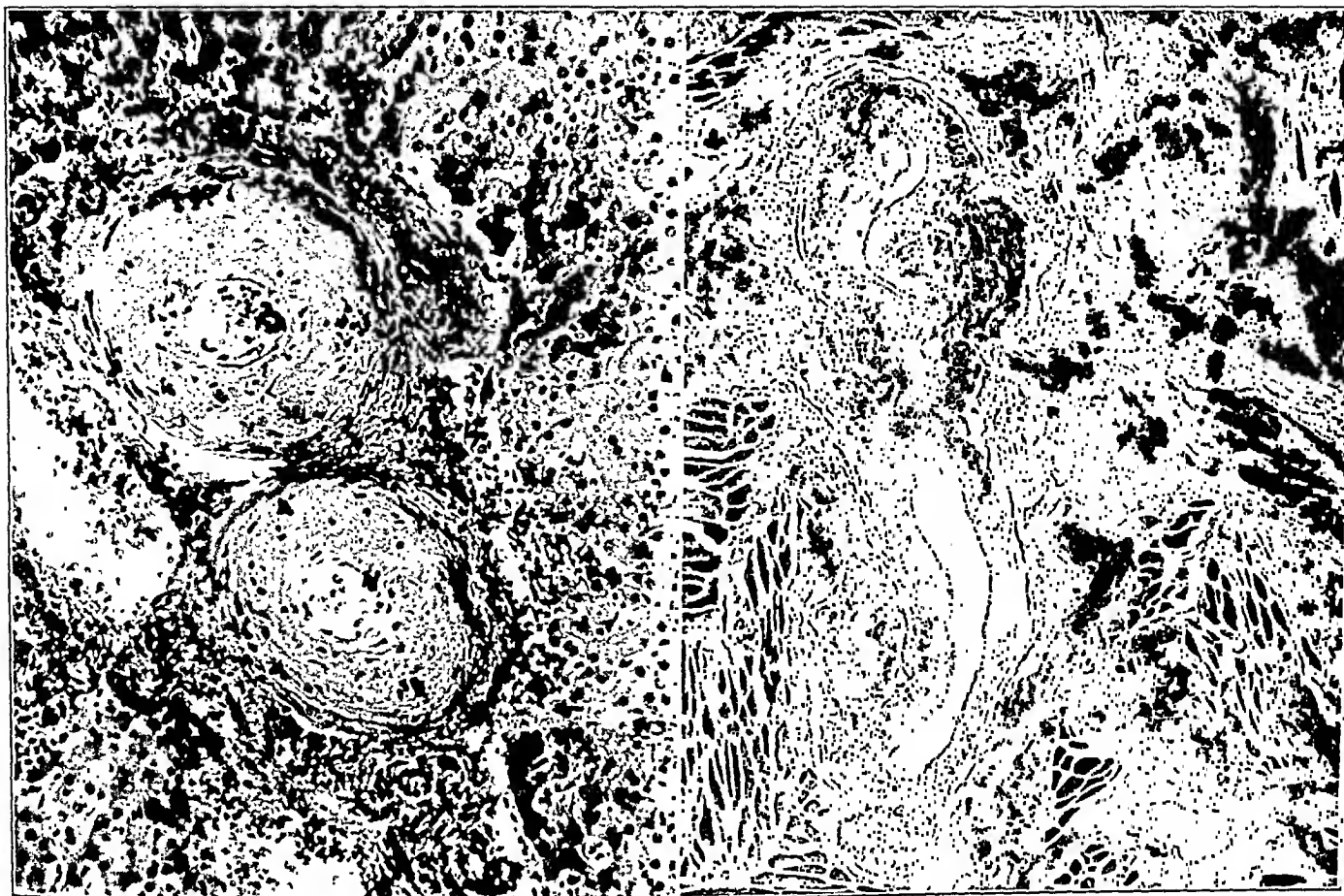


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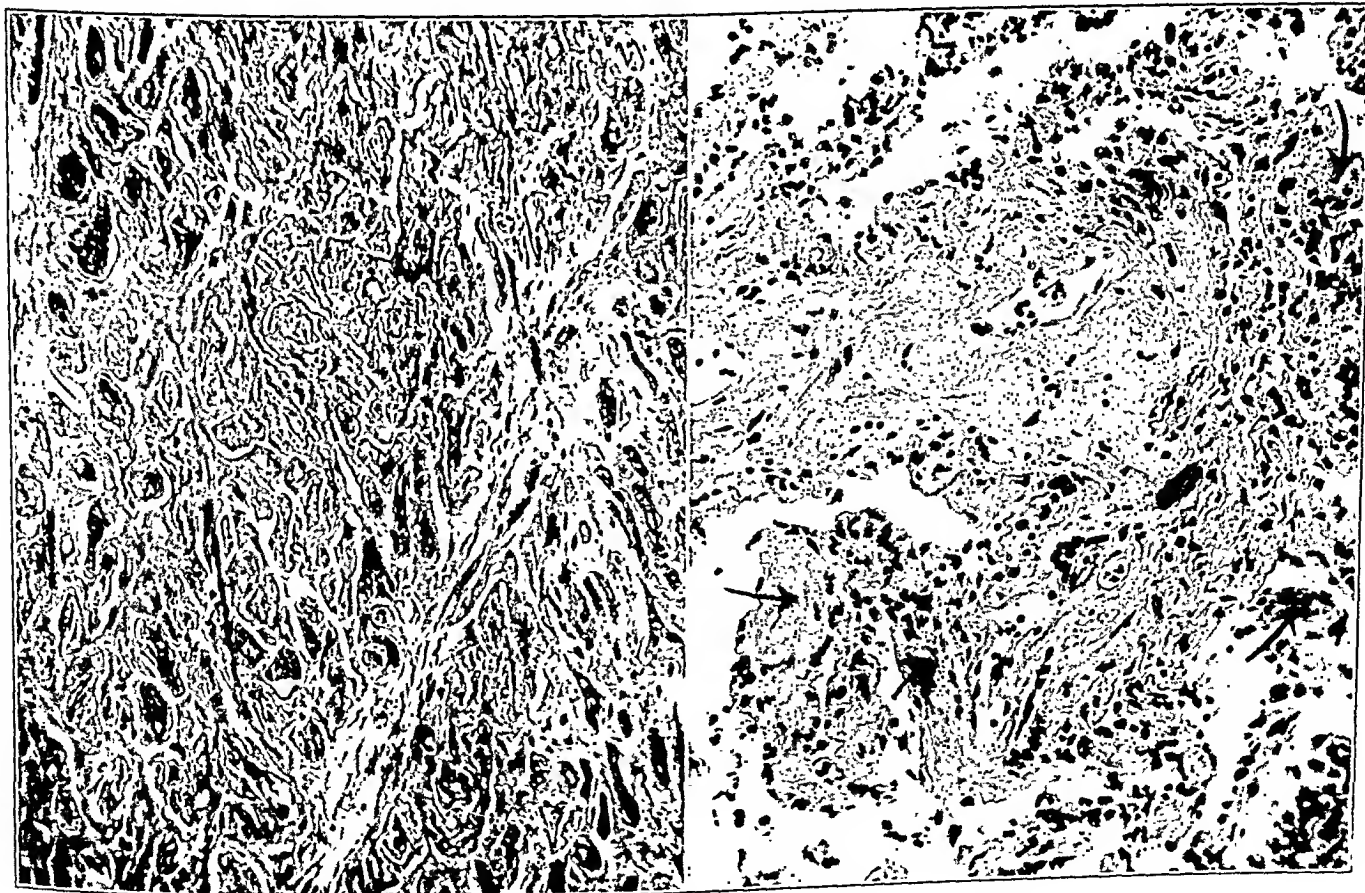
PLATE 130

- FIG. 4. Arteries of liver showing same changes as those in Figs. 2 and 3. $\times 150$.
- FIG. 5. Tongue. Large masses of amyloid substance replace and displace the muscle fibers. Methyl violet stain. $\times 60$.
- FIG. 6. Heart. Left ventricle stained with azocarmine. There is diffuse atrophy of the muscle due to pressure of a substance which stained like immature collagen; in sections stained with methyl violet the larger deposits also gave a faintly positive reaction like amyloid substance. $\times 250$.
- FIG. 7. Lung. There are large and small masses of homogeneous material, the central portion of which stained like amyloid substance. The arrows indicate plaques or beads of the substance in the alveolar walls. Hematoxylin-eosin stain. $\times 300$.



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SPONTANEOUS RUPTURE OF THE PULMONARY ARTERY *

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Spontaneous rupture of the pulmonary artery implies a break in the continuity of the vessel wall which occurs without evidence of trauma, aneurysm, or gross extrinsic pathological changes.

Among the extrinsic causes of perforation of this large vessel are those producing erosion of the wall from the external surface, such as tuberculosis, inflammation, or malignant changes in the surrounding structures. Fittje ¹ reported a case of a 34 year old male where death was due to hemorrhage caused by erosion of a necrotic gumma into the pulmonary artery. Clerc, Bascourret and Froyez,² Bonte,³ and Sternberg ⁴ described ruptures of luetic aortic aneurysms into the pulmonary trunk.

Traumatic ruptures of the pulmonary artery may be caused by crushing of the thoracic cage, stab wounds, gunshot wounds and so on. Marble and White ⁵ reported death in a young army officer from multiple pulmonary hemorrhages 5 months after a gunshot wound in the chest. Autopsy showed a traumatic aneurysm of the right pulmonary artery with a valve-like opening connected with a bronchus.

Henschen,⁶ Posselt,⁷ Wahl and Gard,⁸ and D'Aunoy and von Haam,⁹ in a thorough search of the literature up to 1933 collected 87 cases of pulmonary aneurysm involving some portion of the trunk or its two main branches. In 10 of these cases death was due to rupture of the aneurysm. Two were of the dissecting type. A dissecting aneurysm is not a true aneurysm in the usual sense, but is formed by the escape of blood into the wall of a vessel leading to the separation of the coats. Dissecting aneurysms of the aorta have been reported many times,^{10,11,12} and 9 cases have been found in 6800 autopsies by the Department of Pathology of Stanford University. Spontaneous rupture of a vessel may lead to the formation of a typical dissecting aneurysm unless the rip is so complete that death ensues immedi-

* Supported in part by the Rockefeller Fluid Research Fund of the School of Medicine of Stanford University.

Received for publication June 8, 1935.

ately. On the other hand, a dissecting aneurysm may be the initiating factor in a complete rupture. These two conditions are similar, are probably the results of the same underlying pathological processes, and the two terms are often used interchangeably.

The occurrence of a spontaneous rupture of the pulmonary artery with slight dissection and fatal hemorrhage into the pericardium is so rare that a search of the available literature reveals only 2 cases^{13,14} at all similar to the following.

REPORT OF CASE

Clinical History: Lane Hospital No. 167579. The patient was an American widow, 44 years of age, who gave no history of rheumatic fever or chorea. There had been a normal pregnancy at the age of 18. At 28 she developed congestive heart failure with ascites and edema. After 6 weeks bed rest she continued to work for 9 years. Another brief period of failure occurred at 37, and a more severe one at the age of 38 years, when she was brought into Lane Hospital. Her condition during this attack was reported because of the development of a curious heart rhythm.¹⁵ Although continually disabled, she did not have medical care from her 38th until her 44th year, when she was again brought into the hospital, stuporous, deeply cyanotic, with severe dyspnea and markedly distended neck veins. Ascites and liver enlargement had been present for years. On her first hospital entry the liver was large and pulsating, but 6 years later it was hard and easily movable. She improved during 15 days in the hospital but died in her sleep.

Physical Examination: The heart was huge, and by X-ray had a "mitral" shape, with slight dilatation of the aorta, and a prominent pulmonic arch. The rhythm was regular except for the episode occurring during her first hospital entry.¹⁵ There was a loud diastolic murmur at the apex and in the second and third left interspaces close to the sternum; no systolic murmur was present. The apex beat moved with respiration and change of chest position. The blood pressure was 130/90. Although the neck and facial veins were distended, they pulsated very little. The absence of orthopnea over a 6 year period in this deeply cyanosed patient with a huge heart, large liver and distended veins was most remarkable, as also was her continued activity for 16 years after the first onset of congestive failure.

Laboratory Data: The electrocardiogram was that of right axis deviation with P waves broad and notched. Blood, urine and Wassermann tests were not remarkable.

Diagnosis: Mitral stenosis. Tricuspid insufficiency was suggested by several examiners, but none mentioned tricuspid stenosis.

AUTOPSY REPORT

An autopsy (No. XXXVI-397) performed 12 hours after death showed the body of a small, well developed, poorly nourished female of about 45 years of age, weighing about 100 pounds. Livor mortis

was marked, as was cyanosis of the head, neck and shoulders, with the neck veins distended to 2.25 cm. in width. A moderately soft nodule 3 cm. in diameter was palpable on the right side of the neck apparently attached to the thyroid gland. The chest was flat and symmetrical. The abdomen was moderately distended with fluid. The extremities were wasted and free of edema.

Both pleural cavities were free of adhesions, but the left contained 200 ml. and the right 1500 ml. of thin, slightly bloody fluid. The striking finding was the pericardium distended with 1000 ml. of dark clotted blood. The heart chambers were collapsed, except for a markedly distended right atrium filled with clot similar to that in the pericardium. The venae cavae were each dilated to 4.5 cm. in diameter and filled with clot. The pectinate muscles of the right atrium were hypertrophied to 0.6 cm. in diameter. The tricuspid valve measured 10 cm. in circumference about the base, but the cusps were moderately thickened with fibrous tissue and adherent, forming a rigid, circular stenotic opening 1.5 cm. in diameter and 5 cm. in circumference (normal 12 cm.). The right ventricle was markedly dilated and formed half the apex; the muscle was firm and hypertrophied to 0.9 cm. at the base, with large papillary muscles 1 cm. in diameter. The pulmonary valve was normal, 7 cm. in circumference. The pulmonary artery measured 9 cm. in circumference 1 cm. above the valve; the wall was 0.15 cm. thick and the intima was roughened by scattered atheromatous plaques, the largest 1.2 cm. in diameter. The left branch measured 7.5 cm. in circumference and the right 6.5 cm. with similar atheroma. *In the left lateral wall of the pulmonary trunk was a fresh, jagged, longitudinal separation of the intima and a portion of the media measuring 7 cm. in length extending from the base of the valve upward toward the left branch through one of the atheromatous patches* (Fig. 1). Two cm. above the valve the rupture was complete with a 1 cm. opening through the adventitia and pericardium. Here the adjacent tissues were slightly hemorrhagic. The lateral separation of the media extended only 1.5 cm. from the tear in its widest dissection. The whole appeared to be fresh as though the result of an instantaneous rupture. The left atrium was moderately dilated and the muscle hypertrophied to 4 mm. The mitral valve measured 7 cm. about the base, the cusps were adherent and fibrous, forming a rigid circular orifice 2.5 cm. in circumference (normal 10 cm.). The left ventricle was normal in

size, muscle slightly flabby, 1 cm. in thickness at the base, with no visible scarring. The aortic valve was normal, 6.5 cm. in circumference. The aorta measured 7.5 cm. in circumference 1 cm. above the valve, with slight atheroma at the base, but the arch was normal. There was also slight atheroma of the thoracic and abdominal aorta and of the coronary arteries. The heart with short lengths of the great vessels weighed 435 gm.

Both lungs contained small apical scars. The right was almost completely collapsed. The left was pink and air-bearing. Neither showed a noticeable increase in fibrous tissue. The large branches of the pulmonary artery were moderately atheromatous.

A moderately soft, globular, well encapsulated tumor 2.5 cm. in diameter projected laterally from the right lobe of the thyroid gland.

The peritoneal cavity contained 2000 ml. of clear, pale yellow fluid. The abdominal viscera were markedly congested. The spleen weighed 450 gm. The left kidney was hypoplastic, measured 7.5 by 2.5 by 2.5 cm., and weighed 35 gm. The right kidney measured 13 by 5.5 by 4 cm. and weighed 210 gm. Both showed old flat infarct scars. There was a slight cervical erosion and a small subserous fibromyoma of the uterus. In addition to marked congestion the gastro-intestinal tract revealed a healing puckering ulcer in the mid-portion of the lesser curvature of the stomach, and dark brown pigmentation of the large bowel (melanosis coli). The liver measured 25 by 18 by 8 cm. and weighed 1650 gm. The surface was roughened by numerous nodules varying from 0.3 to 3.5 cm. in diameter and cut with considerably increased resistance. The cut surface showed a marked passive congestion and atrophy with extensive fibrosis, also dilatation of the portal veins. The adrenals and pancreas were normal.

MICROSCOPIC EXAMINATION

Histological sections of all organs were examined. Summaries of the more pertinent are as follows:

Sections of the pulmonary artery showed the tear extending through all layers including the pericardium (Fig. 2). The slight lateral dissection was between the fibers of the outer fifth of the media which were spread apart by the blood clot (Fig. 3). Small, typical atheromatous intimal plaques were present. The media contained irregular spaces filled with an acellular, homogeneous-staining

mucoïd material which disrupted the normal pattern of elastic fibers, muscle and fibrous tissue (Fig. 4). The nuclei were absent in the tissues surrounding these spaces, and many fat droplets were present. The muscle was decreased in amount, the elastic fibers were farther apart, some thicker than normal, others frayed and fragmented. Fibrous tissue crowded the elastic fibers apart and replaced them in moderately large areas, especially near the adventitia. The adventitial and subpericardial tissue near the rupture was heavily infiltrated with red blood cells. Small areas of early thrombus formation showed marked fibroblastic proliferation with many round cells, plasma cells and scattered eosinophiles. The arteriolar walls of the adventitia and outer media were thickened by intimal proliferation so that the lumens were more than half occluded in some instances and the venules were distended with blood. There was slight round cell infiltration of the vasa vasorum. The picture suggested that the pathological process had been present in the outer wall of the pulmonary artery prior to the fatal rupture. Giemsa, acid-fast, and spirochete stains revealed no organisms.

A section through one of the larger intimal plaques in the pulmonary artery away from the line of rupture showed rather marked intimal thickening due to acellular hyalinized fibrous tissue with fatty degeneration, necrosis and cholesterin crystals extending into the media. The media was thinner, with fewer elastic fibers and more definite breaks in continuity beneath the plaque than in other areas (Fig. 5). The adventitia was normal except for marked congestion.

Sections of the liver typified varying degrees of "cardiac cirrhosis" ranging from dilatation and congestion of the central veins and sinusoids, with atrophy and apparent increase in central lobular fibrous tissue, to areas of marked fibrosis with obliteration of the lobules, leaving scars causing depressions in the liver capsule with intervening hypertrophic nodules of liver tissue. The periphery of the nodules was not invaded by cellular fibrous tissue as in the ordinary type of nodular cirrhosis.

The thyroid nodule was a well encapsulated, fetal type of adenoma. The stomach showed a benign fibrosing ulcer.

The lungs were normal except for slight edema and scattered areas of hemosiderin-laden phagocytes. There was no material increase in fibrous tissue. The walls of the arterioles were normal but

the intima of the larger arteries showed thickened fibrous plaques with fatty changes.

Anatomical Diagnoses: Chronic endocarditis with mitral and tricuspid stenosis and insufficiency; marked right-sided cardiac hypertrophy and dilatation; slight dilatation of the pulmonary artery; moderate pulmonary sclerosis; fatal rupture of the pulmonary artery with hemopericardium; marked "cardiac cirrhosis"; generalized passive congestion; fetal adenoma of the thyroid; healing ulcer of the stomach; hypoplasia of the left kidney; old infarcts of both kidneys; melanosis coli; endocervicitis; fibromyoma of the uterus; and healed apical pulmonary tuberculosis.

DISCUSSION OF LITERATURE

The etiology of spontaneous ruptures and dissecting aneurysms of large vessels has long been in dispute, and the theories as to the mechanism of their development are varied. Since these conditions are practically limited to the aorta, the theories have evolved about their occurrence in this vessel. A typical dissecting aneurysm begins in the ascending portion of the aorta with a transverse tear through the intima and outer two-thirds of the media with blood dissecting between the medial layers. The splitting may involve the entire aorta and extend into the iliac arteries. Commonly the blood ruptures externally into the pericardium, pleura or mediastinum with death of the patient, but it may rupture back into the lumen, or may heal, and the patient may live normally for many years.¹⁶ Spontaneous rupture is a term best reserved to describe those cases of sudden death due to the bursting of a large vessel with very little gross evidence of the cause of the fresh tear and but slight dissection. The fundamental processes of these two conditions are probably the same.

Laennec¹⁷ reported primary rupture of an atheromatous intima as the cause of dissection in the aorta. Peacock¹⁸ was unable to produce the lesion in normal aortas by increased pressure in the lumen but dissection occurred when the intima was damaged transversely to the long axis of the vessel. In 1852 von Rokitsky¹⁹ emphasized degenerative changes in the media and since that date many writers have favored medial changes with high blood pressure and primary rupture of the intima as essential factors. The medial damage has varied from inflammatory changes with destruction of the

elastic fibers to minute hemorrhages, tears and muscular atrophy. Lifvendahl²⁰ reported three cases of spontaneous rupture of the aorta with uniform changes consisting of an intimal tear at the base of the aorta, high blood pressure, renal arteriosclerosis and syphilitic mesaortitis. He emphasized the absence of extensive gross changes in the aorta but found considerable microscopic destruction. Tyson¹² in 1931 reviewed the many theories and in reporting 5 cases, among which were 3 with intact intimal lining of the aorta, agreed that dissecting aneurysm is dependent upon degenerative changes of the medial coat, probably due to obliteration of a large number of vasa vasorum from arteriosclerosis or a low grade inflammatory process. He postulated the formation of a hematoma, formed by rupture of one or more vasa vasorum, which split apart the medial fibers with secondary intimal tears.

The aortic and pulmonary trunks are histologically quite similar, but it is a fact that atheroma, syphilis and aneurysm are common in the aorta and rare in the pulmonary artery. Possibly the natural differences in blood pressure and the variations in carbon dioxide and oxygen content of the blood in each are pertinent.

DISCUSSION OF CASES

Duffield¹³ made no suggestion as to the etiology in his case of dissecting aneurysm of the pulmonary artery. This was a 50 year old female with pulmonary and tricuspid insufficiency, right heart dilatation and hypertrophy, dissecting aneurysm of the right pulmonary artery the size of a "duck's egg," containing fibrinous clot, aneurysmal dilatation of the left pulmonary artery without dissection, advanced atheromatous degeneration and hemopericardium.

The case reported by Durno and Brown¹⁴ was that of a 33 year old male who died in his sleep. The pulmonary artery with its two main branches and a patent ductus arteriosus were uniformly dilated, the walls showing large plaques of atheroma. At the bifurcation of the pulmonary artery the intima and media were torn through, forming a small dissecting aneurysm which had burst into the pericardium. The right ventricle was markedly hypertrophied. They explained the etiology by atheroma and high blood pressure caused by the patent ductus arteriosus.

The mechanism of rupture in our case can only be conjectured. The tear must have caused almost instantaneous death from heart

tamponade. The sharp edges of the tear and the slight lateral dissection indicated a fresh rupture which must have been the result of high pulmonary arterial pressure and weakness of the vessel wall. The etiology of the medial and adventitial changes may have been the partial obliteration of the vasa vasorum, either by localized arteriosclerosis or the inflammatory process responsible for the valvular stenoses, with the resultant degeneration of the wall of the pulmonary artery as seen in the microscopic sections. Along the line of the medial adventitial junction small areas of early thrombus formation with marked fibroblastic proliferation and round cells suggested that hemorrhages had occurred there prior to the fatal rupture. Tyson's theory of hematoma formation before the rupture of the inner wall may have played some slight part in this picture.

The marked tricuspid stenosis with the resultant hypertrophy and dilatation of the right atrium and vena cava undoubtedly gave origin to the large pulsating liver observed several years previous to the patient's death, and also to the smaller, firm nodular liver found at autopsy. In gross and histologically, the liver showed a marked "cardiac cirrhosis" type V of Lambert and Allison.²¹

These 3 cases are similar in that all showed dilatation of the pulmonary trunk with atheroma, hypertrophy of the right side of the heart, and fatal hemorrhage into the pericardium through rupture of the pulmonary artery. No microscopic examinations were reported in the first 2 cases.

Clinical and Pathological Correlations

King²² first noted that tricuspid insufficiency due to distention of the right ventricle in mitral stenosis led to a decrease in pulmonary congestion by the reflux into the atrium and systemic veins. Tricuspid stenosis obviously has a similar or even more marked effect on decreasing the blood flow into the lungs. This probably explains the absence of orthopnea in the patient in spite of the very high systemic venous pressure and also why she was able to live 16 years after her first attack of heart failure with such severe valve lesions. In this case the clinicians failed to recognize the tricuspid lesion from its effects on the circulation, even though the position of the diastolic murmur suggested such a lesion to one examiner. It should be re-emphasized that tricuspid injury must be considered in a patient with chronic endocarditis who has intense cyanosis or venous en-

gorgement without orthopnea and with relatively little dyspnea when at rest. In such a case the absence of vigorous pulsation of neck veins points to a physiological stenosis, not insufficiency. Although the clinical result of the tricuspid lesion is to diminish pulmonary inflow, in this case there was definite pulmonary hypertension as a result of the mitral lesion. But the gross changes in the pulmonary artery were slight and the sudden separation of the intima, in this case as in many cases in which the aorta is involved, cannot be explained merely by the chronic lesions of the artery which precede it, or by pulmonary hypertension. Just as thousands of aortas are subjected to severer stress or have more advanced degenerative lesions than the one that develops dissecting intramural lesions, so also hundreds of pulmonary arteries, subjected to higher pressure and higher pulse pressures by patent ductus or pulmonic fibrosis, withstand these stresses even after marked atherosclerosis has developed.

The areas of the valve openings, in this case, were for the tricuspid 1.8 cm., pulmonic 3.9, mitral 3.9, aortic 4.3. The rigid mitral and tricuspid valves obviously must have been open during systole. As the pressure in the large arterial trunks is higher than in the auricles, a mitral opening nearly as large as the aorta must have permitted about as much blood to flow back into the auricle and pulmonary veins as entered the aorta at each beat. Curiously enough, there was no systolic apical murmur, which indicates that one cannot gauge mitral insufficiency by the heart sounds, or recognize pure stenotic lesions by the faintness of the systolic as contrasted with the diastolic apical murmur. Even with some pulmonic hypertension, the pulmonary pressure is much lower than the aortic, and with systolic apertures in the auriculo-ventricular valves bearing the same relation to arterial openings, reflux into the right auricle will be less than on the left. Here the tricuspid valve was less than half as large as the pulmonic, so that systolic reflux was less than half as great into the right auricles as into the left, while the impediment to diastolic inflow was twice as great. The anatomical findings point, therefore, to a tricuspid lesion which was physiologically stenotic in its effects.

SUMMARY AND CONCLUSIONS

1. A case of sudden death from a spontaneous rupture of the pulmonary trunk in a female 44 years of age who had suffered periods of marked cardiac decompensation for 16 years is presented.

2. Gross changes in the pulmonary trunk were inadequate to explain the rupture, but microscopic alterations of a degenerative nature were rather marked. These in combination with increased pulmonary arterial pressure must be considered as the cause of the rupture.

3. The tricuspid stenosis probably explains the absence of orthopnea, and also the fact that the patient lived so many years in spite of chronic heart failure and cyanosis.

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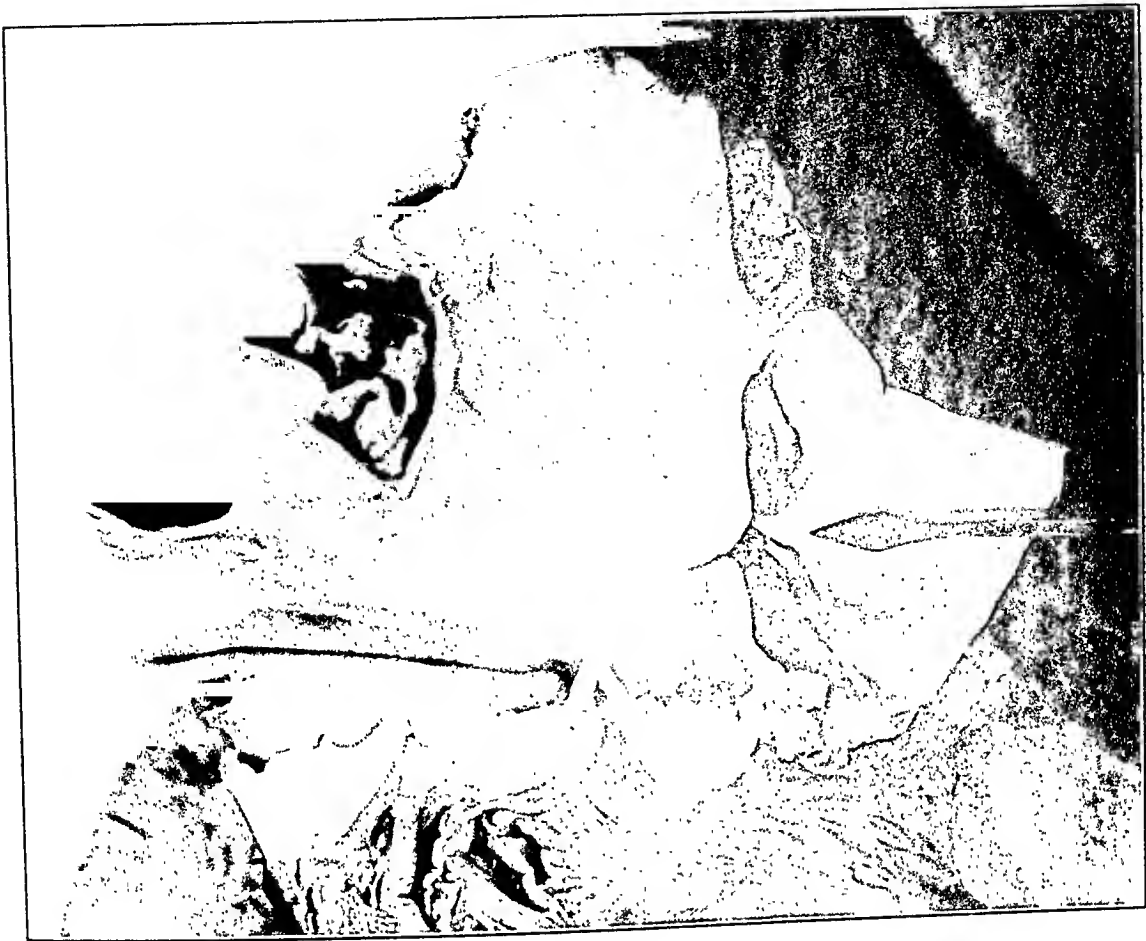
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DESCRIPTION OF PLATES

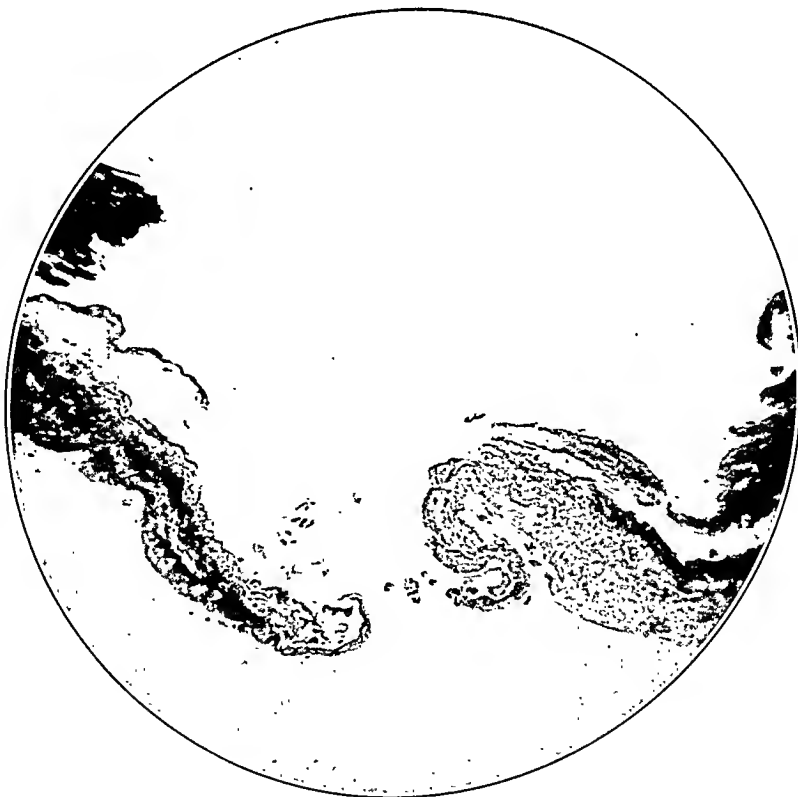
PLATE 131

FIG. 1. Photograph of the pulmonary trunk showing the longitudinal rupture extending from the valve into the left branch.

FIG. 2. Photomicrograph of the tear extending through all layers of the pulmonary trunk and pericardium. Elastic tissue stain. $\times 10$.



I



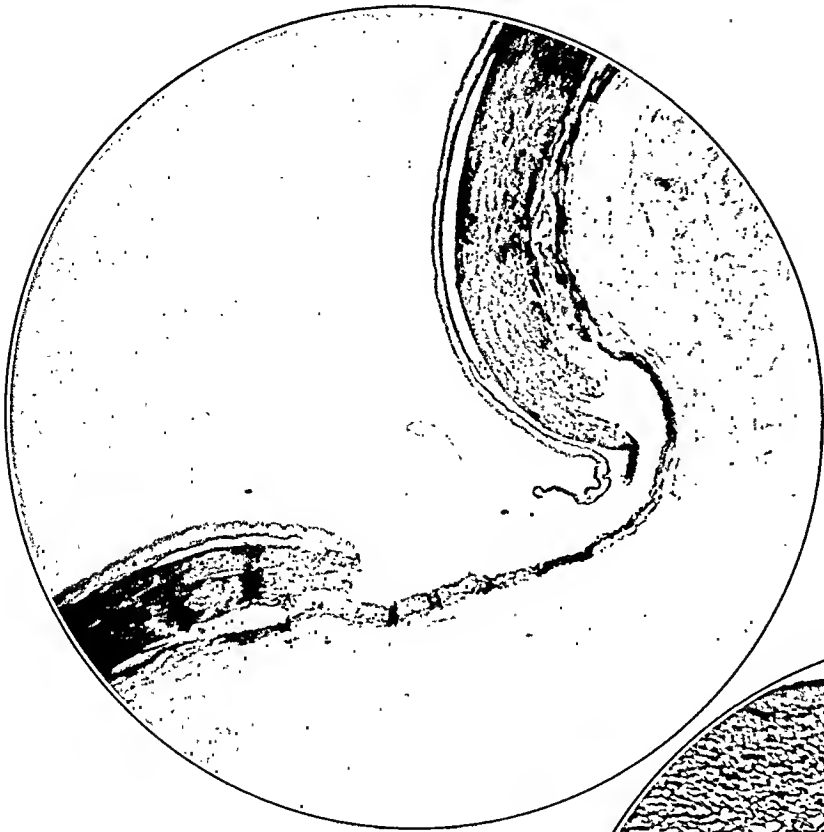
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PLATE 132

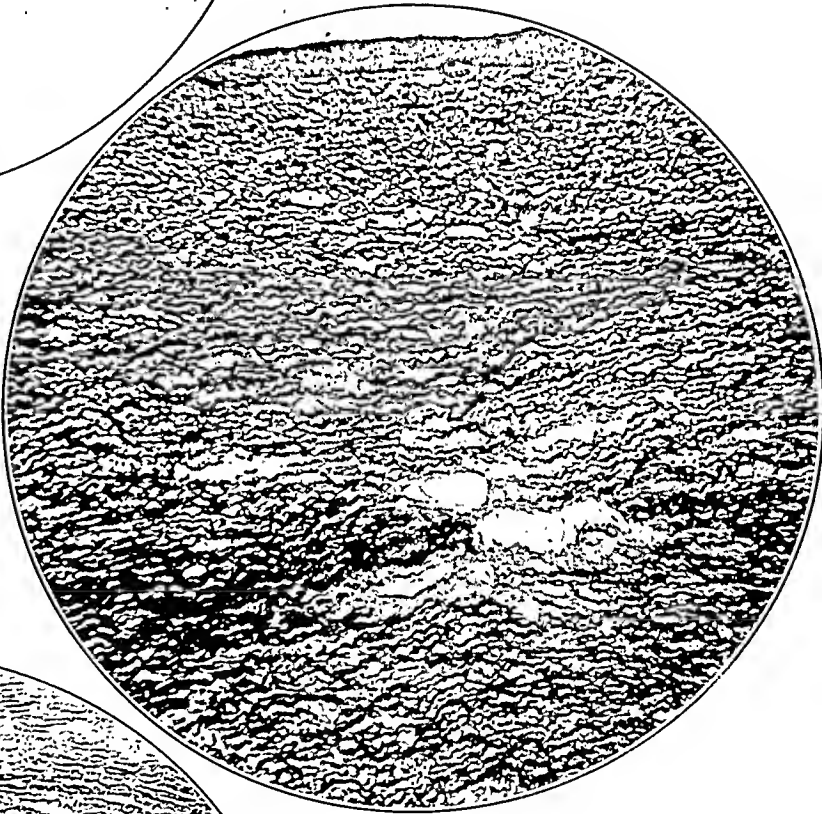
FIG. 3. Photomicrograph of the tear through the intima and inner four-fifths of the media with lateral dissection. Elastic tissue stain. $\times 10$.

FIG. 4. Photomicrograph showing irregular spaces filled with acellular mucoid material which disrupts the normal pattern of the media. Elastic tissue stain. $\times 80$.

FIG. 5. Photomicrograph showing marked intimal thickening with thinning and breaks in the continuity of the media of the pulmonary trunk. Elastic tissue stain. $\times 80$.



3



4



5

NEUROFIBROMA OF THE PHARYNX ASSOCIATED WITH VON RECKLINGHAUSEN'S DISEASE *

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Von Recklinghausen's disease, frequently referred to as neurofibromatosis, is characterized by multiple tumors of the nerves, mollusk-like skin tumors, pigmentation of the skin and elephantoid thickenings, isolated or in groups. The disease picture is frequently associated with other malformations and anomalies such as epispadias, cryptorchidism, uterus bicornis, polythelia, malformation of the kidneys, bones or muscles, and dwarfism or infantilism.¹

According to Ewing² the cause of neurofibromatosis is not known, but it is assumed that such lesions are the result of a congenital malformation of the ectoderm, which under any one of a great variety of exciting causes may slowly or rapidly develop one or more of the manifestations of the disease. The work of Hoekstra³ on the inheritance of this disease supports the idea of a dysontogenetic origin.

Primary pharyngeal neurofibromas developing either as a manifestation of von Recklinghausen's disease or as solitary tumors are extremely rare. Primary tumors of the oral cavity or pharynx arising from the nervous system or containing nerve elements are also rare. Neurofibromas developing about the face and neck most frequently occur in association with generalized neurofibromatosis and may secondarily bulge into the oral cavity.

Fibrous tumors are not common in the oropharynx and are to be distinguished from those in the nasopharynx. Those in the oropharynx are usually described as simple fibromas but they are probably never pure fibromas, the fibrous tissue usually being mingled with fat cells in variable amounts.⁴

New and Childrey⁵ made a study of 357 cases of tumors of the tonsil and pharynx observed in the Mayo Clinic in a period of 14 years, from 1917 to 1930 inclusive. In this series no mention is made of neurofibroma, yet, their series contains 2 fibromas, 1 fibromyxoma, 2 fibrosarcomas and 1 fibromyxosarcoma.

The term neurofibroma is a descriptive term and seems to be ap-

* Received for publication June 20, 1935.

plicable to the tumor nodules found in von Recklinghausen's disease. Because of the varying mixture of nerve fibers with connective tissue the term neurofibroma is descriptive in the sense that it is a fibroma on and in a nerve, the fibers of which contribute toward the formation of the tumor. In retaining for the tumors of von Recklinghausen's disease the name neurofibroma, the term must be understood to signify a tumor that contains both nerve fibers and connective tissue. It is not a new growth of nerve tissue, although there are nerve fibers and apparently new nerve collaterals running in it. It is not a simple fibroma but is a fibrous connective tissue reaction that is a part of a more general process.

A pure neurofibroma, as seen in von Recklinghausen's disease, is in one sense not a neoplasm at all.⁶ There are wandering nerve fibers derived from the involved nerve and a surrounding tangle of reactionary connective tissue which is a magnification of the widespread pathological alteration of the nerves in the system disease. Confusion arises from the fact that at times within these neurofibromas true neoplasms such as perineurial fibroblastoma may appear and may grow so large as to displace most of the neurofibroma tissue to the periphery. In von Recklinghausen's disease, however, nerve fibers will be found to enter each tumor with few exceptions, while in solitary perineurial fibroblastomas the comparatively normal nerve is invariably applied to the capsule of the tumor without penetrating it.

Grossly a neurofibroma is usually attached to a nerve. It is most frequently somewhat rounded and nodular in shape, firm in consistence and, on section, white and often almost translucent.

The histological picture is characterized by palisading and parallelism of the nuclei and a tendency to form nuclear eddies and streams. The nuclei are usually elongated and irregular. They may be large, however, and contain a condensation of chromatin which resembles to some extent the nucleoli of nerve cells, especially in degenerating areas.

Some authors attempt to differentiate perineurial fibroblastoma from true neurofibroma in that the former does not show nerve fibers. The nerve or nerve root to which the tumor is attached may be found at the periphery of the tumor running on or in the capsule, and at times a spiral coat ganglion may be dragged out by the nerve root and flattened over such a fibroblastoma.

A few cases of tumors associated with nerves located in the pharynx and adjacent structures have been reported in the literature. Spiess⁷ reported what was apparently the first case of ganglioma arising in this location. This tumor arose in the subglottic region of the larynx. The upper part was visible between the vocal cords. Microscopically the tumor was composed of nerve tissue — a fibrous basis with numerous ganglion cells which had all the staining properties and characteristics of nerve cells. It is the opinion of the writer that gangliomas do not have the same histogenetic origin as neurofibromas and should not be classed with the type of tumor under consideration.

Colledge⁸ in 1930 reported a case of a neurofibroma associated with von Recklinghausen's disease in a woman 44 years of age. The tumor presented itself as a firm swelling occupying the right aryepiglottic fold and pyriform fossa and entirely concealing the right vocal cord. It could not be palpated externally. Microscopically it proved to be a pure fibroma. One can not say definitely that there is any relation of this tumor to the generalized neurofibromatosis.

Forbes⁹ in 1925 reported a case of plexiform neuroma (ganglion neuroma) associated with von Recklinghausen's disease in a girl 14 years of age. Oral examination showed a large, irregularly smooth mass occupying practically half of the faucial pharynx and involving the right tonsillar pillar. The left half of the pharynx was not involved. Grossly the specimen consisted of nine pieces of tissue; the largest measured 30 by 23 by 8 mm., and the smallest 10 by 5 by 4 mm. All pieces were irregular in shape, pale pink in color, semi-soft in consistence for the most part and loose in structure with coarse, papillary projections on the free surface. On section there were white and translucent streaks throughout, suggesting fine, twisted nerve trunks. Meeker,¹⁰ who reported the case from a pathological viewpoint, was unable to find any record of another similar tumor involving the pharyngeal mucous membrane. She considered this a true neuroma, a manifestation of von Recklinghausen's so-called neurofibromatosis.

Askanazy¹¹ reported a solitary nerve tumor of the post-pharyngeal wall which he, after ten years of study, finally designated as a "neurinoma Verocay," the modern designation of many of the tumors of von Recklinghausen's neurofibromatosis.

Figi¹² reported a solitary neurofibroma of the pharynx in a woman

61 years of age. This case is unique; I am unable to find a report of another similar one. Physical examination revealed extreme prominence of the left tonsil. It was firm and bulged almost to the median line of the pharynx, apparently because of the marked tonsillar enlargement. There was a palpable mass high in the left cervical region. At operation the tonsils were found to be uniform in size, the bulging on the left being due to a large, firm, somewhat irregular mass situated externally to the aponeurosis. Upon removal of the tumor it was found to be encapsulated with projections on its surface extending laterally and inferiorly. It was firm in consistence and measured approximately 4.5 by 6 by 8 cm., and on section was yellowish in color. Microscopically the tumor was reported to be a degenerating neurofibroma. Through a communication with Dr. Gordon B. New¹³ I understand that he has recently removed a neurofibroma similar in location, size and structure from a young girl.

Suchanek,¹⁴ Holmgren¹⁵ and Vail¹⁶ each reported a case of Schwannoma of the larynx, but no mention was made as to the involvement of the pharynx.

The writer is reporting a case of neurofibroma of the pharynx associated with von Recklinghausen's disease which is similar in type and location to the ones reported by Forbes⁹ and Askanazy.¹¹ The case reported by Figi¹² was a solitary neurofibroma of the pharynx without the usual skin manifestations of von Recklinghausen's disease.

REPORT OF CASE

Clinical History: A white woman, 44 years of age, was admitted to the Paterson General Hospital on April 25, 1934. She complained of a lump in her throat and progressive difficulty in swallowing and breathing for the past few months. Family and past history were not remarkable.

Physical examination revealed a growth in the pharynx, somewhat elliptical in shape, measuring about 8 by 10 cm. It occupied the left half of the pharynx and extended from the eustachian tube above to the epiglottis below. The tumor bulged anteriorly and obstructed a view of the larynx.

Over the chest, abdomen, back and extremities were many soft and firm, pedunculated and sessile tumor masses, some measuring as much as 2 by 1 by 1 cm. Associated with the tumor masses were pigmented areas of the skin varying in size from 1 to 2 cm. (Fig. 1).

On April 26, 1934, under general anesthesia, a firm tumor mass apparently fixed to the side and back of the pharynx was removed in two pieces. The larger piece measured 7.5 by 6 by 2.5 cm., the smaller 4 by 2 by 4 cm.

Pathological Report: (No. 34-380). Grossly the specimen consists of seven pieces of firm tissue, weighing together 50 gm. The largest piece measures 5 by 2.5 by 2 cm. All pieces are firm in consistence and, on section, white in color with a smooth cut surface. The outer surface of the larger pieces is nodular, irregular and covered with a dense fibrous capsule. Microscopically the characteristic histological features are the palisade-like and parallel arrangement of the elongated nuclei. The nuclei also tend to be arranged in streams, eddies and whirls. Running parallel to the long axis of the nuclei are long parallel fibers of collagen. In some areas the tumor is rather cellular, the cells are a little larger and some of the nuclei are hyperchromatic. Very rarely a mitotic figure is found (Fig. 2).

Diagnosis: Neurofibroma with an occasional mitotic figure.

On April 30, 1934, two of the tumor masses were removed from the chest.

Pathological Report: The specimen consists of two pieces of tissue. The larger is 1.5 by 1 by 0.4 cm. and is covered on one surface with skin. On the skin surface is a slightly elevated, soft tumor mass 1 cm. in diameter. The second piece is a pedunculated, firmer tumor mass measuring 0.4 by 0.4 by 0.2 cm.

Diagnosis: Neurofibromatosis.

The patient made an uneventful recovery and showed no signs of recurrence of the pharyngeal tumor at the end of 12 months.

SUMMARY

1. A report of the successful removal of a neurofibroma weighing 50 gm. from the pharynx of a woman 44 years of age who presented a typical picture of von Recklinghausen's disease is made.
2. The cause and anatomical structure of neurofibromatosis, as found in von Recklinghausen's disease, is discussed.
3. Only 3 other cases of neurofibroma of the pharynx were found in the literature.

NOTE: I am indebted to Dr. N. P. Lobsenz for his permission to report this case and for his assistance in obtaining the clinical data.

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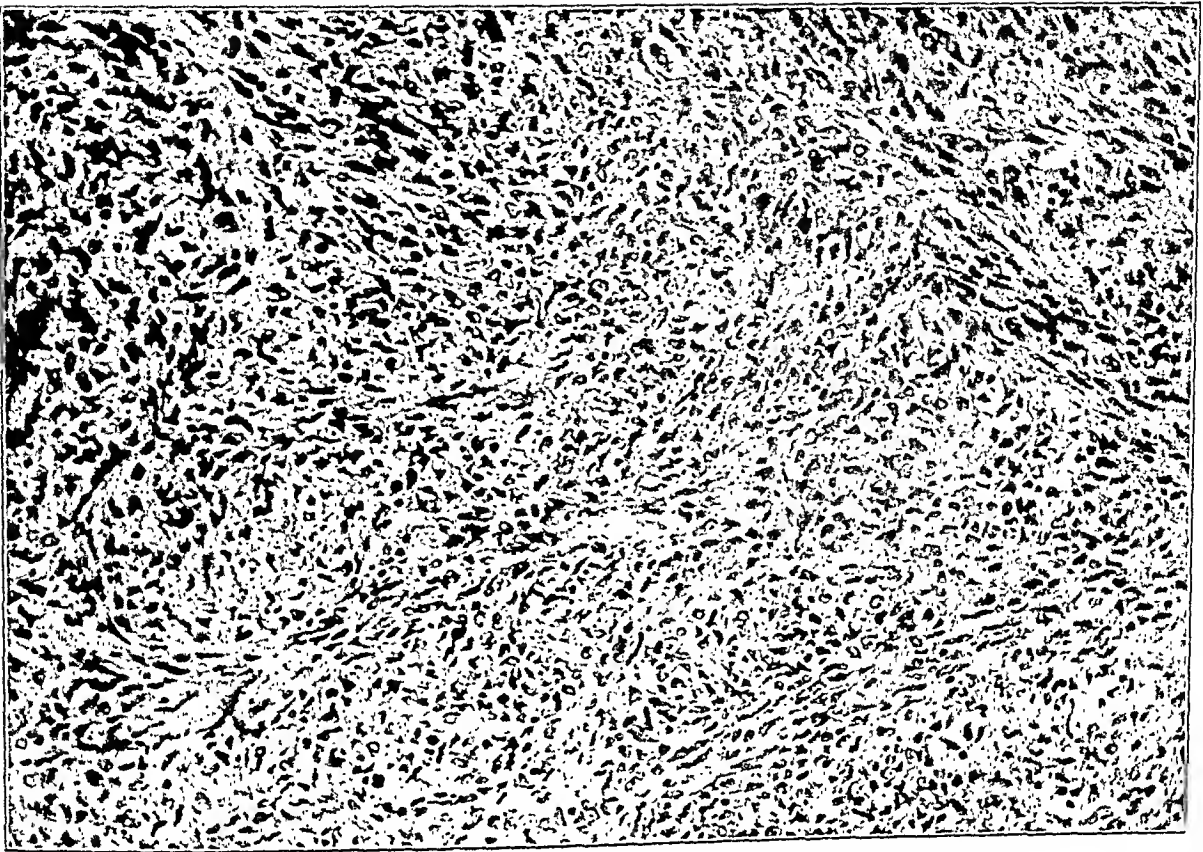
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DESCRIPTION OF PLATE

PLATE 133

FIG. 1. Photograph showing neurofibromas associated with areas of pigmentation (von Recklinghausen's disease).

FIG. 2. Photomicrograph showing the most cellular area of the neurofibroma from the pharynx. $\times 120$.



2



1

Neurofibroma of Pharynx

Davis

A TECHNIQUE FOR DEMONSTRATING THE PERIVASCULAR NERVES OF THE PIA MATER AND CENTRAL NERVOUS SYSTEM *

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In the course of a study of the nerves of intracranial blood vessels it was found that the Cajal reduced silver method and the methods employed by Stöhr and by Bielschowsky would at times demonstrate vascular nerves on the pial and dural vessels. But no such nerves could be demonstrated on intracerebral vessels. The method of Gros-Bielschowsky likewise stained nerves on all but the intracerebral vessels until it was modified as will be described below. It was only due to the persistence and versatility of our technician Edward Dockrill that a reliable method was at last devised which serves to demonstrate nerve fibers both on pial and intracerebral blood vessels.

The addition of acid to the formaldehyde fixative is the most important new element in the procedure. Different acids may be used but the best results were obtained with a combination of formalin and citric acid, which we have called Dockrill's fixative. Thick sections may be made but best results were obtained when the whole vessel dissected free from the brain was carried through the various solutions on a glass rod.

The routine procedure is as follows:

1. Wash out blood from the material to be used by perfusion of saline through the blood vessels, or by rinsing uninjected tissue in saline.
2. Fix, preferably by injection, with 10.5 per cent citric acid (powder is more readily soluble but crystals may be used) in 20 per cent commercial formalin (neutrality of the formalin used is not important). If injection is not possible leave the material 2 or 3 days in fixative before staining. Otherwise stain shortly after injection.
3. Prepare tissue by dissecting out blood vessels under a dissecting microscope. The pia is cut and turned slightly backwards so as

* Received for publication June 28, 1935.

to expose the vessel entering at right angles to the brain. Brain tissue is then carefully pushed away from around the vessel until a sufficient length is obtained when the vessel is cut and lifted out. Intracerebral vessels of the anterior perforated space and large branches of the middle cerebral artery, such as the lenticular striate, are the most readily dissected.

4. Wash blood vessels or sections in 2 changes of distilled water and place them in a 20 per cent aqueous solution of silver nitrate for 2 hours.

5. Pass material through 4 changes of 20 per cent formalin. Use Petri dishes for this and have about 100 cc. of solution in each dish. (It is a good plan to have both the formalin and the subsequent materials laid out ready for use before beginning this step; see schema below.)

6. Pass directly from 20 per cent formalin to ammoniacal silver nitrate, prepared by adding concentrated ammonia (28 per cent) drop by drop, to a 20 per cent solution of silver nitrate. A precipitate forms which redissolves on addition of more ammonia. Add about 3 drops in excess. If the material turns black or becomes too dark, cautiously add more ammonia drop by drop until the concentration is right as seen by the tissue. Examine the vessel or section under the microscope for degree of staining. The nerves will be seen to "come up" slowly.

7. When the degree of staining is sufficient place for 1 or 2 minutes, according to thickness, in 20 per cent ammonia water.

8. Wash in distilled water acidified with 12 to 15 drops of glacial acetic acid. Any trace of alkalinity precipitates gold chloride from solution; that is the reason for the acidified water wash.

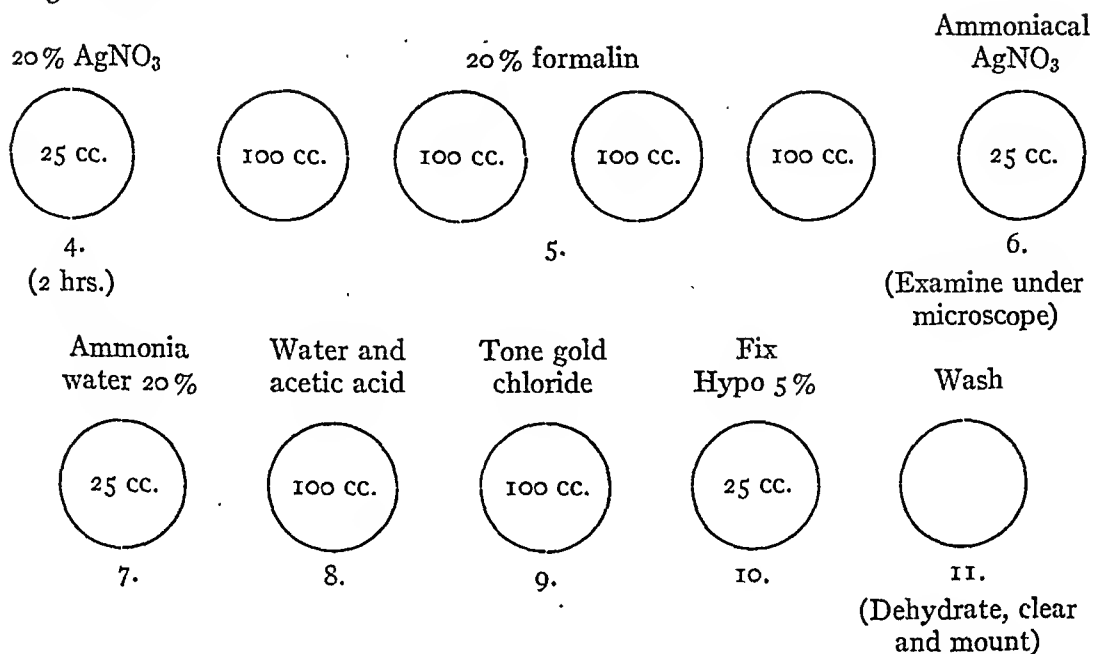
9. Tone in 0.2 per cent (1:500) yellow gold chloride for $\frac{1}{2}$ to 1 hour.

10. Fix in 5 per cent sodium thiosulphate 15 minutes.

11. Wash in water. Dehydrate in 3 changes of 95 per cent alcohol and mount in Canada balsam from carbol-creosote-xylol mixture.

SUMMARY

1. Wash.
2. Fix.
3. Dissect.



It often happens that insufficient staining of nerves is obtained because of precipitate forming before staining is complete, or because the material darkens too rapidly in the ammoniacal silver bath. Such material can be decolorized and then, after refixation, stained again. Two solutions are used for this, each kept in a separate bottle. The first is a 10 per cent solution of iodine in a 20 per cent aqueous solution of potassium iodide. The second, a 10 per cent aqueous solution of potassium cyanide, is added drop by drop to the iodine until it clears. When the stained vessel or section is placed in this it becomes almost instantly colorless. Wash in water and leave in fresh fixative for 2 or more days before restaining. This procedure will often "bring up" nerves that have previously failed to show and one may repeat the process as many as a dozen times on the same material. Care should be exercised in using it, however, as the solution liberates hydrocyanic acid fumes.

Preparations made as described above seem to last indefinitely as those now 5 years old have not changed. The nerves on the intracerebral vessels are continuous with those upon the pial vessels and nerve endings may be impregnated at times.^{1, 2}

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PAPILLOMATOSIS PERITONEI *

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A benign, branching villous papilloma, the surface cells of which are continuous with the mesothelium of the peritoneum, is of considerable rarity since we can find no record of a similar case in the available literature, nor have we found others familiar with the condition.

In the case to be reported the patient's medical history and death apparently have no relation to the peritoneal papillomas. However, a brief summary is presented.

A 70 year old judge had been in excellent health, never having suffered from any serious disease, but there gradually developed over a period of 1 year an increasing difficulty in voiding. The symptom progressed to complete urinary retention during the last 2 weeks of life. There was a moderately enlarged prostate but no other important physical abnormalities, except for an occasional extrasystole. The urine showed a cloud of albumin and many pus cells. Blood chemistry, Wassermann test and other routine laboratory procedures were negative. A suprapubic cystotomy was performed as the first stage of a prostatectomy and there was some attempt at repair of a urinary bladder diverticulum. The patient remained in apparently good condition throughout the operation. The pulse, respiration and blood pressure taken 4 hours postoperatively (5 minutes before death) were normal and the patient gave no evidence of impending sudden death.

The important findings at autopsy included a heart weighing 460 gm. with badly sclerosed coronary arteries but showing no myocardial scarring or evidence of myocardial degeneration. There were moderate congestion and edema of the lungs, congestion of liver, spleen and kidneys, obstructive benign prostatic hypertrophy, chronic prostatitis, acute cystitis with a large diverticulum in the fundus of the bladder and many small papillary tumors on otherwise normal peritoneal surfaces. The opened intestines contained

* Received for publication May 20, 1935.

no sign of neoplasm and no single primary source of the peritoneal lesions could be found.

The papillomas were scattered over the parietal peritoneal surfaces, being concentrated for the most part on the diaphragm, omentum and mesentery of the small intestine. They varied in size from slight irregularities, invisible to the unaided eye, to lesions nearly a centimeter in diameter and occurred singly or in small groups. Occasionally there was a small villous bridge between adjacent lesions. The smallest lesions were irregular elevations of the peritoneum with distinct increase in the size and number of mesothelial cells and with a change to cuboidal shape. A very slight increase in cellularity of the underlying connective tissue was noted. A few lesions were found as microscopic crypts below the surrounding peritoneal surface. Simple finger-like villi less than a millimeter in length were frequent. Microscopically they may have had many small buds covered by small cuboidal cells which showed no mitotic figures. In the largest lesions heavy villi branched from a common center at the top of a rather long and narrow pedicle. The main villi may have branched once or twice and small buds were located anywhere on these but more particularly on the smaller branches. The larger villi tended to be heavy and club-shaped and their mesothelium thin and flat, approaching the normal. The connective tissue was coarse and had comparatively few cells in the pedicle and proximal portions while the ends of villi showed fewer cells and very delicate fibrils. A few well formed blood vessels were found even in the small branches. Nerve fibers could not be demonstrated. The small numbers of lymphocytes present in a few of the older villi could hardly be considered the result of inflammation of bacterial origin.

An attempt was made to learn more of this tumor by direct correspondence. Dr. James Ewing's first impression was that the papillomas were epithelial implantations from some ruptured epithelial cyst. If no such primary tumor could be found, then by exclusion one must conclude that they were endothelial in origin and represent true fibro-endothelial papillomas. Except for multiple cystic tumors of the peritoneum, he had never seen anything like the case in question.

Dr. William Boyd felt that the condition was a papillomatosis of the peritoneum, but using the word merely to indicate a proliferative lesion of connective tissue cells projecting from the surface and not

necessarily an epithelial neoplasm, and although he had never seen or heard of papillary growths resulting from chronic irritation of the peritoneum, this was a possible etiological factor.

Dr. Arthur E. Hertzler believes the condition to be a primary, benign, wart-like growth of the peritoneum, the only one of its kind in his experience. A number of other pathologists and surgeons were of a similar opinion.

The tendency of certain embryonic derivatives of the peritoneum to form papillary tumors of a somewhat similar type as the above is a generally recognized phenomenon. It seems unusual that papillary tumors of peritoneum are not more frequently described.

SUMMARY AND CONCLUSIONS

An unusual and widespread villous type of benign peritoneal papilloma was an incidental finding at the autopsy of an elderly man dying 4 hours after the first stage operation for prostatectomy. The tumor is considered primary in the peritoneum and related to similar neoplasms in structures derived from the peritoneum.

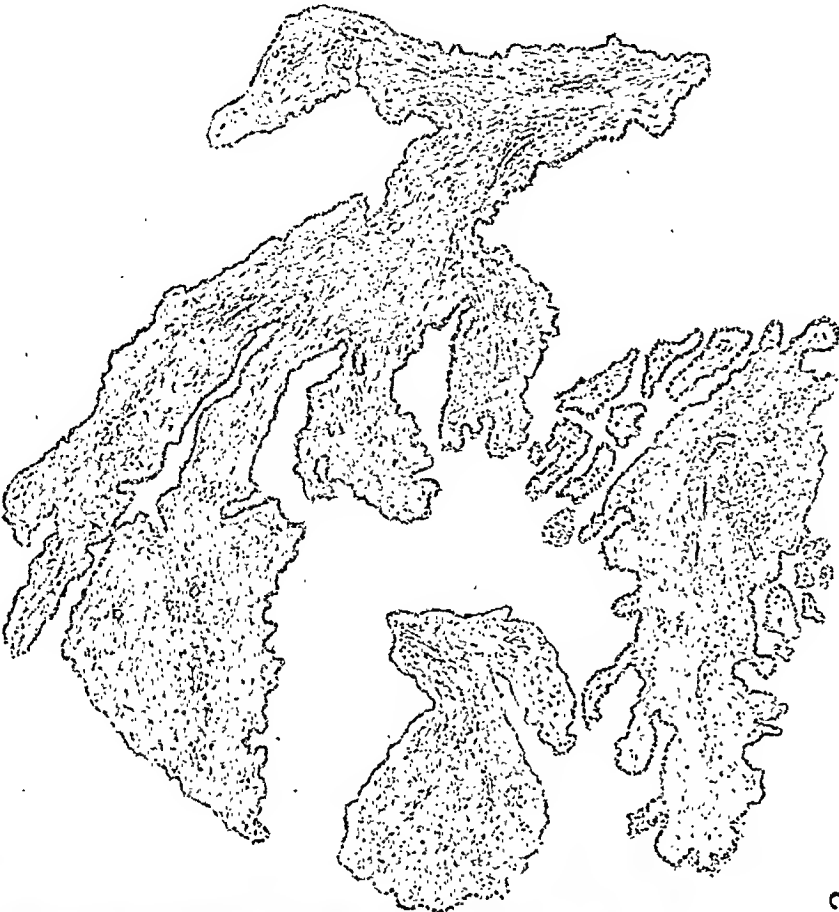
DESCRIPTION OF PLATE

PLATE 134

FIG. 1. Mesentery of small intestine covered with benign papillomas. (Inset)
A group of lesions of varied sizes. $\times 5$.

FIG. 2. Branching villi and "buds" from a large lesion. $\times 50$.

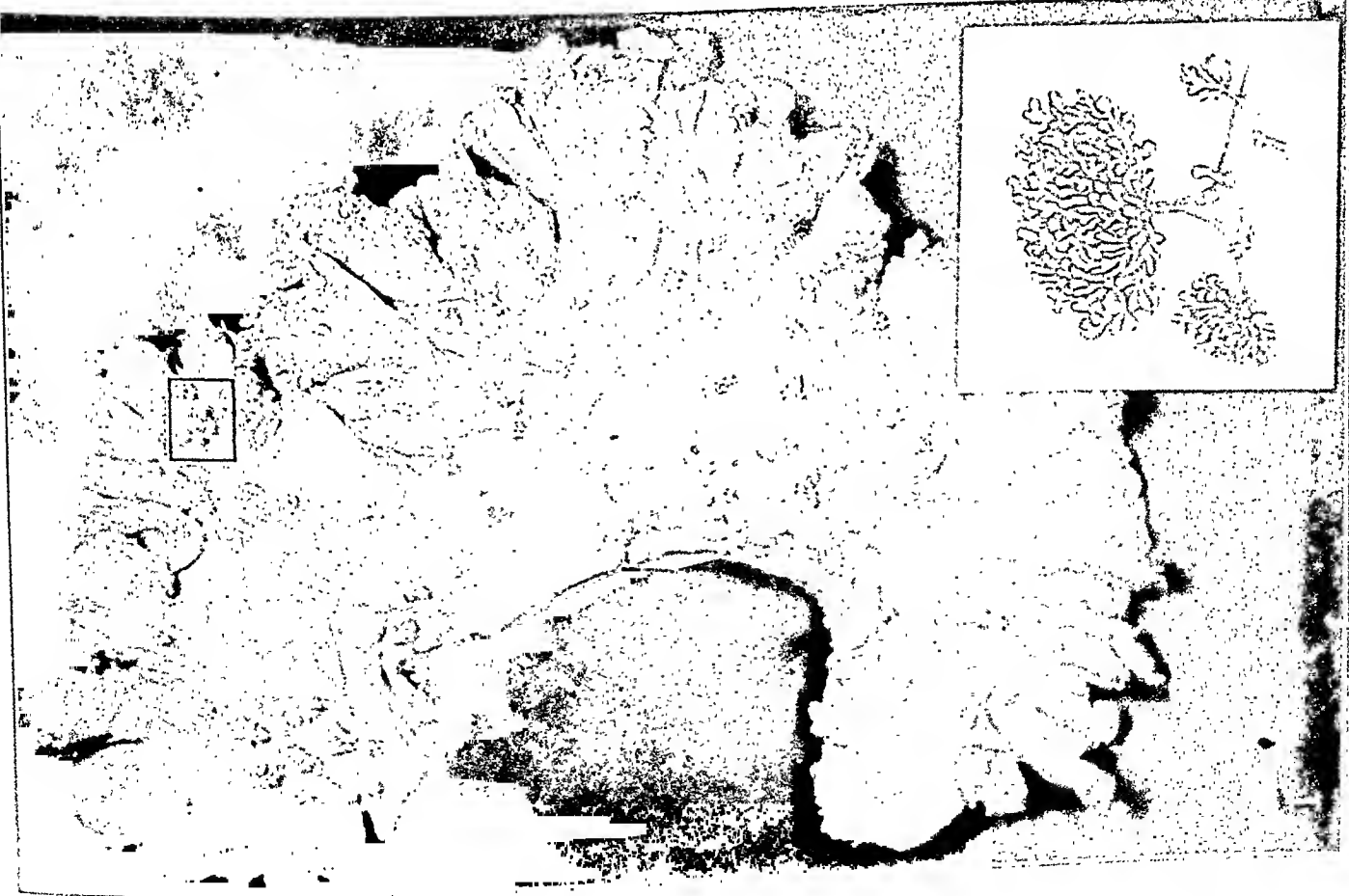
FIG. 3. Early lesion showing relation to mesothelium. $\times 250$.



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Papillomatosis Peritonei

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